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PREVENTION OF OVINE MASTITIS BY THE USE OF STAPHYLOCOCCUS TOXOID.

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MASTITIS in sheep may be caused by bacteria of different species. Thus, from time to time, the following have been reported to be associated with the disease: staphylococci, streptococci, coliform bacteria, *C. pyogenes,* pasteurella, or *Cl. welchii,* by E. Joest (1920), Leyshon (1929), Miessner and Schoop (1932), Marsh (1932), Weinberg, Forgeot and Moureu (1937). In Germany it was first shown by Dammann and Freese (1907) that "infectious mastitis" in sheep is due to a Gram-negative pleomorphic bacillus resembling that first described by M. Christiansen under the name of "Bacterium purifaciens" but which, according to Haupt (1932), is distinguishable from it serologically.

This paper deals with the staphylococcus form of the disease, concerning which there are a number of reports in the literature.

Long ago, Nocard (1887) showed that the acute mastitis of gangrenous type—the so-called "black udder" or "black garget," or, in German, "Euterbrand"—is caused by relatively minute staphylococci and he suggested that "soluble poisons" formed by the organisms were largely responsible for the lesions and for the animal's death. Nocard's observations were directed towards establishing the cause of an acute mastitis which was troubling the owners of hand-milked ewes kept for the manufacture of Roquefort cheese. In these flocks, considerable losses had been reported annually; not rarely, 10 per cent. of the sheep were said to be attacked, with many deaths and those which recovered being rendered useless for milk production. Bridré (1907) also refers to the losses arising in this class of flock through mastitis, the average case incidence being put at 5 per cent. and the case mortality at 20 per cent., though at times enzootics were reported with a case incidence as high as 20 or 25 per cent. Detroye (1906) mentions an outbreak in a flock of 45 sheep. Four animals were affected and the udder secretion from one of them showed staphylococci on microscopical examination.

Gangrenous mastitis has often been reported from other countries besides France. In Germany, the disease was described by Kotelmann as early as 1836 and later by Esser (1889), Huth (1894) and Sahm (1901) but in none of these cases apparently was the diagnosis confirmed bacteriologically. Pfeiler (1908) witnessed the disease among a number of ewes which had lambed six to eight weeks previously and its severity seemed to increase as the outbreak progressed. Cultural examination of the secretion revealed an organism identical with that described by Nocard. It was noted that a similar outbreak had occurred in this flock two years previously. Stephan and Geiger (1921) gave an account of an outbreak of mastitis of acute malignant type affecting about 100 sheep of which 70 per cent. died. Staphylococci were isolated from a number of the affected sheep. No attempts at prevention were made since the outbreak was subsiding by the time an exact diagnosis was made. Cases with similar clinical features had occurred during the previous year and most of those affected died. Miessner and Schoop (1932) made a bacteriological examination of 27 cases of ovine mastitis. The commonest infecting agent was the Dammann-Freese organism but staphylococci were responsible for three cases and were present in four others.

Gilruth (1910) encountered the disease in New Zealand, though it was stated to be comparatively rare. He showed that, although cases might be clinically mild, the staphylococcus isolated might be highly " virulent " when injected by the teat canal into the udders of experimental sheep.

In a private communication, Mr. R. J. Roe, F.R.C.V.S., informs me that in Cyprus acute gangrenous mastitis is quite common amongst sheep, usually during the first few weeks of lactation, and is also well known among goats. In that island the sheep are raised very largely for milk production, the ewes being milked twice daily from one to six months after lambing. In consequence, their udders are highly developed. The incidence of the disease varies greatly from year to year, but there are occasional outbreaks affecting up to 20 per cent. or more of the ewes in a flock and sometimes in several flocks in one village area.

Mostarda (1937), in Italy, writes of an outbreak of gangrenous mastitis with 20 cases in a flock of 150, but apparently no bacteriological examinations were made. Altara (1933) and Pegreffi (1934) refer to a pustular dermatitis of the udder seen among sheep and goats in Sardinia, with mastitis as an occasional complication. Pegreffi concluded that the disease was due, not to a filtrable virus, but to a staphylococcus with characters similar to those of *Staph. aureus*. Rosati and Bertolino (1938) describe staphylococcus mastitis among sheep and goats in Sicily. In some localities the disease is said to appear almost every year, especially in winter and spring and chiefly among animals in the first lactation. The mortality reaches 50 or 60 per cent.

Klesov (1936) reports the occurrence of ovine staphylococcus mastitis in the Ukraine where the incidence in lactating ewes is said to range from 7·8 to 15 per cent. annually, with 70 to 80 per cent. case mortality. During 1934-5 he studied 25 cases from all of which a staphylococcus, identical with that described by Nocard, was isolated in pure culture.

In Great Britain, according to Pool (1926), gangrenous mastitis causes a great deal of loss in some parts of the country every year. According to information supplied to Leyshon (1929), mastitis is prevalent in the Eastern Counties and is stated to cause a number of deaths annually. The incidence is said to vary greatly in different flocks but tends to be higher following a spell of cold weather. One farmer reported that in one year in his flock of

394 animals there had been 68 cases, of which 43 were fatal. During Leyshon's investigations no actual outbreak was encountered but he was able to examine the milk bacteriologically from 38 sporadic cases in ten flocks. From 36 of these the organism responsible was isolated ; in 15 cases staphylococci were present in large numbers and in pure culture and in nine others staphylococci were mixed with other organisms (streptococci in six of the nine cases). Nine of the 15 pure culture cases had died and four were destroyed, while four of the nine mixed culture cases had died—in three of these four, staphylococci were the predominant organisms. All the staphylococcus strains liquefied gelatin and all but one fermented mannite.

Finally, it may be remarked that gangrenous mastitis is also well known among goats, reports of the disease being available from Germany (Kossmag, 1933, 1936) and Italy (Cubeddu, 1935 ; Rabaglietti, 1934).

In the matter of prevention, Bridré (1907) showed experimentally that sheep could be actively immunized by a number of subcutaneous inoculations of living staphylococci, in the sense that the later injections were well tolerated, compared with the earlier ones. Serum from such immunized sheep largely prevented the injurious effects arising from the infusion of living staphylococci through the teat canal, and sheep which had previously been given living staphylococci subcutaneously were similarly protected. In field experiments an " attenuated " vaccine was first used. This was prepared by leaving cultures for three or four months at 37° C., 1·0 c.c. distributed at two sites being given subcutaneously to sheep during the first two months of gestation. In one experiment mentioned, 755 ewes were vaccinated and 1,150 left as controls ; among the former there were 11 cases of mastitis as compared with 54 cases among the controls. The incidence had thus been reduced from 4·7 to 1·45 per cent. In further trials, Bridré (1913) used a " sensitised " and heat-treated vaccine as a means of obviating local effects due to the inoculum. Staphylococcus suspensions were heated at 56° C. for an hour, centrifuged, and the sediment treated with serum from immune sheep ; after further centrifuging the sediment was then washed and resuspended in saline at a certain concentration. An experiment is quoted in which 245 sheep were vaccinated and 1,015 controls left untreated ; among the former there was one case of mastitis (0·41 per cent.) whereas among the controls 34 cases (3·35 per cent.) of the disease appeared.

Klesov (1936) tried as a vaccine 48-hour broth cultures of staphylococcus strains isolated from cases of mastitis, after the cultures had been sterilised by the addition of formalin to 0·4 per cent. The vaccine was first tried under experimental and then under natural conditions. In a flock of 491 sheep, 72 were vaccinated once (group 1), 89 twice (group 2), and 103 three times (group 3), leaving 227 as controls. The vaccinations were begun in June and by the following August the incidence of mastitis had been : group 1, four cases, of which two died ; group 2, five cases ; group 3, one case ; controls, 21 cases, of which 11 died. By a triple vaccination therefore the percentage incidence had been reduced by 9·25 to 0·97.

Rosati and Bertolino (1938) also used a formol vaccine, prepared in a similar manner to that of Klesov, in nine flocks with some 2,200 sheep, of which 310 were affected. Two doses were given at a week's interval to both the affected and apparently healthy sheep. The mortality in infected sheep prior to vaccination was 45 per cent. ; after the first dose of vaccine the number of new cases fell to 23 and after the second injection to five, while only three of the 310 cases died.

Experimental Results.

Now that it is known that one of the ways by which pathogenic staphylococci produce their effects is by the formation of filtrable toxins and that these can be converted into toxoids by means of formalin, it was obvious that toxoid should be tried as a means of preventing gangrenous mastitis in sheep. Should it prove of value in this respect, the results ought to be applicable to similar forms of mastitis in cows and goats.

During the winters of 1936-37 and 1937-38, Mr. C. V. Watkins (Seale-Hayne Agricultural College, Devon) vaccinated a number of ewes in flocks where from previous experience the disease might have been expected to appear, leaving an equal number of unvaccinated controls. Unfortunately, the main object of these experiments was not attained. The first winter 25 ewes were inoculated subcutaneously, each with three doses of toxoid (2, 5 and 5 c.c.), prepared from three staphylococcus strains of animal origin, but there was only one case of mastitis in a control ewe. The second winter 250 ewes belonging to four flocks were each given two doses of toxoid (2·5 and 5·0 c.c.), but no cases of mastitis were reported in any of the sheep. The first two doses were given during the period of gestation, at about an interval of three weeks, and the second injection was timed to fall as near as possible a fortnight before lambing was due. Some of the animals were given a third dose of toxoid (5·0 c.c.) about a month after lambing. The toxoid was prepared from toxins having about three Lh doses of α toxin per c.c.* In the 1936-37 experiment, the sheep were bled before the first dose and ten days after the third dose of toxoid, the control sheep being bled at the same time. Titrations of serum from the treated sheep in this laboratory showed increases of antitoxin from four to 24 times (average ten times) the original titre.

In view of these results, it became necessary to test the value of toxoid under experimental conditions at this Institute and with this object five experiments have been made.

Methods.

The formalinised toxoid used in these experiments (batch K772) was prepared in the Wellcome Physiological Research Laboratories from a toxin containing about three Lh doses of α toxin per c.c. and using a staphylococcus (Taylor 2) isolated from a case of gangrenous mastitis in a cow. In experiment 5 an alum toxoid was used, prepared from batch K772 by addition of 2·0 per cent. potash alum. This amount of alum gave a product with 7·5 Lf doses per c.c. and an N content of 0·065 gm. per cent., or 11·5 Lf doses per mg. N.

Except in the case of the alum toxoid used in experiment 5, the toxoid was always mixed with an equal volume of saline at the time of use. Injections were made subcutaneously, and, when two doses were given, at an interval of three weeks.

* The term "Lh dose" refers to the amount of toxin neutralised by one unit of standard antitoxin.

Titrations of serum for antitoxin were made using (1) a toxin having an Lh dose of 0·125 c.c. This toxin was prepared in March, 1937; stored on ice without preservative its strength had remained practically constant for over two years. (2) a standard serum (KCP2256) having 580 units of α antitoxin per c.c.

The sheep sera were heated at 56° C. for 30 minutes and stored without preservative. They were titrated with the toxin at Lh, Lh/10 or Lh/50 levels as necessary, the toxin being mixed with serum in amounts falling by 50 per cent. differences. So far as possible, sera taken on different dates during an experiment were tested on the same day and under precisely similar conditions.

The test inoculations on vaccinated and control sheep were made by instilling into one or both sides of the udder through the teat canal a mixture of staphylococcus " toxin " and culture (strain Taylor 1), the inoculum being then lightly massaged into the tissues. (At the time of isolation, Taylor 1 strain, which had been isolated from a case of gangrenous mastitis in a cow, was very virulent for mice and its virulence had been still further enhanced with the aid of mucin before it was used in these experiments). If any secretion was present in the gland, most of it was removed before the inoculation. The " toxin " was prepared by expressing the juice from a soft (0·5 per cent.) agar culture of the staphylococcus which had been incubated for 36 hours at 37° C. in an atmosphere containing 25 per cent. CO_2. The juice was centrifuged to remove most of the cocci before use. The culture was a 24-hour broth culture of the staphylococcus, and, unless otherwise stated, the Taylor 1 strain was used.

Experiment 1 (*February,* 1938) was of a preliminary nature. Three sheep were selected from a group of nine on the basis of the " natural " antitoxin content of their serum, Nos. A and B having less than one unit per c.c. and No. C. having four units. Sheep D, due to lamb and having less than one unit, was added later. The sheep were tested by instilling toxin and culture into one half of the udder, as follows. No. A was given 6 c.c. toxin and the following day was re-injected with 6 c.c. toxin mixed with 1·5 c.c. culture. Nos. B and C were given 6 c.c. toxin mixed with 2 c.c. culture. No. D was given 10 c.c. toxin, prepared from a staphylococcus isolated from a case of chronic staphylococcus mastitis, mixed with 2 c.c. of the culture as used for Nos. A, B and C. In numbers A and B the udder swellings reached the size of a tennis ball, both sheep went off food, became very thin and died seven and twelve days respectively after the injections. Nos. D and C survived, the former giving birth to three lambs 15 days after injection. In both cases the udder swelling reached the size of a cocoanut and was very hard. In this experiment then two of the three sheep with low antitoxin died while the one with larger amounts of antibody survived the test injection.

Experiment 2 (*March to May,* 1938). Thirty sheep were bled and their sera titrated. Six sheep having low titres (average 0·45 units per c.c.) were then selected to serve as controls (Nos. 7 to 12) and six with higher titres (average 2·2 units per c.c.) to be treated with

toxoid (Nos. 1 to 6). It was expected that in the latter the toxoid would act as a secondary stimulus and so provide a good immunity response. The toxoid was given in two doses of 2·5 and 5·0 c.c. at an interval of 22 days. Eleven days later the test inoculation was made on one side of the udder of both vaccinated and control sheep, using 8 c.c. toxin and 2 c.c. culture. The sheep were bled at intervals and the titration results are given in Table 1. On Chart 1 these are reproduced, and on Chart 2 the relative sizes of the udder swellings following the test injection are shown. It will be seen that the toxoid produced a three- to fifteen-fold rise in antitoxin and that in the controls the antitoxin level had risen only slightly, except in No. 10, during the month they were kept before the test injection. After the test injection the amount of antitoxin had increased greatly in the controls and much less or not at all in the vaccinated sheep. The antitoxin increase in fact was a reflection of the extent of the mastitis, and this it will be seen was much less in the vaccinated than in the untreated sheep. This experiment indicated that toxoid treatment can considerably diminish the extent of the mastitis caused by injecting toxin and living culture into the udder.

TABLE I.

EXPERIMENT 2.—UNITS OF α ANTITOXIN PER C.C. SERUM.

Sheep.		*Serum of* 24.3.38 (*before toxoid*).	28.4.38	9.5.38 (*after toxoid*).	19.5.38
Toxoid 28.3.38 and 19.4.38	4	1·2	18·0	18·0	18·0
	2	1·5	9·0	24·0	24·0
	5	1·5	18·0	18·0	12·0
	6	1·5	18·0	36·0	24·0
	1	3·0	18·0	24·0	24·0
	3	4·5	12·0	12·0	18·0
Untreated controls	12	<0·3	1·2	72·0	72·0
	8	0·3	1·2	36·0	36·0
	9	0·3	0·6	48·0	72·0
	11	0·6	1·2	24·0	18·0
	7	0·6	0·6	72·0	72·0
	10	0·6	4·0 *	72·0	36·0

* Staphylococcus (toxin + cult.) injected into udder of all sheep 30.4.38.

Experiment 3 (*June to July,* 1938). It will be noted that in experiment 2 none of the control sheep died, so in experiment 3, which was largely a repetition of experiment 2, the test dose was increased.

Thirty-five sheep were bled* and 12 of them selected as suitable

* One of these sheep had 42 α antitoxin units per c.c. and a chronic staphylococcus mastitis. On the surface of the udder there were several pits which proved to be the ends of sinuses intersecting the gland and containing greenish liquid pus which was extremely rich in staphylococci.

on the basis of the antitoxin content of their serum. The controls (Nos. 19 to 24) had at most 0·8 units α antitoxin per c.c. and those for vaccination (Nos. 13 to 18) had between four and ten units per c.c. The toxoid was given in two doses of 2·5 and 5·0 c.c. at an interval of 20 days. Eleven days later, a mixture of 8 c.c. toxin and 2 c.c. culture was injected into each half of the udder. In No. 14 one half of the udder was "blind," so the full dose of 20 c.c. was introduced into the remaining half. The results of the serum titrations are given in Table 2 and these are reproduced on Chart 3. The relative sizes of the udder swellings are displayed in Chart 4. The toxoid produced a rise in antitoxin ranging from two to eight times the original, while three of the control sheep during the same period had shown a very slight rise. After the test injection the antitoxin increase was similar on the whole in the two groups of sheep, but as in experiment 2 the degree of mastitis was greater among the controls. It should also be noted that in the protected sheep the extent of the mastitis varied inversely with the antitoxin response to the toxoid (*cf.* Nos. 15 and 16). None of the sheep died following the test dose, but control sheep No. 19 had to be killed 20 days later since a large area of necrosis had formed over the abdomen and udder. On *post-mortem* examination the skin over this area was dry and the connective tissues were filled with inspissated pus to a thickness of 2·5 cm. It should be remarked that this sheep had very little natural antitoxin and that the antitoxin response to the test injection was exceptionally low.

TABLE II.

EXPERIMENT 3.—UNITS OF α ANTITOXIN PER C.C. SERUM.

Sheep.		*Serum of* 31.5.38 (*before toxoid*).	30.6.38	12.7.38 (*after toxoid*).	22.7.38
Toxoid 1.6.38 and 21.6.38	13	4·0	32·0	42·0	32·0
	14	4·0	32·0	42·0	42·0
	15	4·0	8·0	42·0	42·0
	17	4·0	10·0	64·0	64·0
	18	8·0	21·0	64·0	64·0
	16	10·0	42·0	64·0	64·0
Untreated controls	19	0·2	0·2	16·0	10·0
	20	0·4	1·3	42·0	42·0
	22	0·4	0·5	64·0	64·0
	23	0·4	1·3	64·0	64·0
	24	0·4	1·3	84·0	84·0
	21	0·8	0·7*	64·0	42·0

* Staphylococcus (toxin + cult.) injected into udders of all sheep 2.7.38.

Experiment 4 (*August,* 1938, *to January,* 1939). The objects were to compare the efficacy of one dose of toxoid with two doses and to obtain some information on the duration of the immunity.

In this experiment it was decided that the initial titres of all animals should be low and as far as possible similar. Sixty-five sheep were bled and 21 animals having titres of 0·8 units or less per c.c. (in all except one) were selected and allocated to three groups which were treated as follows. Animals of the first group (Nos. 31 to 36) were given two doses of toxoid, 2·5 and 5·0 c.c., at an interval of 26 days; those in the second group (Nos. 25 to 30) were given a single dose of 5·0 c.c. on the same day as the first group received its second dose; Nos. 37 to 44 and No. X formed a control group. The treated animals were bled 10, 42 and 89 days after their second or single dose of toxoid and the controls were bled on the same days. Ninety-five days after the toxoid treatment was finished, the test injection of 8 c.c. toxin and 2 c.c. culture was made into one half of the udder. Seven, 20 and 31 days after this injection all the sheep were again bled. All sera taken prior to the test injection were titrated on the same day and all those taken subsequent to this injection were titrated on another day. The titration results are shown in Table 3 and Chart 5. The sizes of the udder swellings are shown in Chart 6. The general and local reactions of the experimental sheep were as follows. Six hours after the injection all the sheep looked sick. The controls took very little food during the first three or four days, the toxoid groups showed inappetence during the first two days only. The day after injection all the controls were noticed to be slightly lame; in the case of No. 41 this persisted for at least three days and in Nos. 39 and 37 during the whole month they were under observation. In the toxoid groups, No. 28, which received one dose of toxoid, was the only sheep noticed to be lame and then only for a day or two.

Control sheep No. X was made very ill by the inoculation and there was a painful œdema round the udder. It remained sprawled out in its pen and was unable to flex its hind legs. On humane grounds it was destroyed on the third day, and the udder on section showed an intense hæmorrhagic inflammation. Two other control sheep, Nos. 39 and 37, showed severe local reactions. In both cases the necrosis due to the injection involved not only the deep tissues of the udder but also the skin, excepting the teat. As a result, the skin became œdematous, purplish and later greenish in colour with hæmorrhagic edges and the swelling was doughy. After 13 days signs of separation at the edges of the necrotic area were noticed and the dried skin over an area 8 to 11 inches by 4 inches, together with a considerable amount of stinking material like inspissated pus, gradually became detached from the underlying granulation tissue. Healing was not complete at the end of a month. Control sheep No. 38 showed changes similar in nature to those of No. 39, but the area of skin involved was not larger than a penny piece.

Among the toxoid sheep, No. 27 (one dose) was the only one to show any necrosis of the skin, but this was extremely slight.

In all cases, the udder swelling was stony hard, and painless

Table III.

Experiment 4.—Units of α antitoxin per c.c. serum.

Sheep.		*Serum of* 6.8.38 (*before toxoid*).	22.9.38	24.10.38	10.12.38 (*after toxoid*).	23.12.38	5.1.39	16.1.39
Toxoid (one dose) 12.9.38	25 ...	0·2	4·0	1·5	1·0	22·5	30·0	22·5
	26 ...	0·3	8·0	4·0	2·0	30·0	45·0	45·0
	27 ...	0·4	6·0	2·0	1·5	30·0	45·0	45·0
	28 ...	0·4	6·0	3·0	1·0	90·0	120·0	90·0
	29 ...	0·8	6·0	3·0	3·0	60·0	60·0	30·0
	30 ...	0·8	12·0	6·0	3·0	60·0	90·0	60·0
Toxoid (two doses) 17.8.38 and 12.9.38	31 ...	<0·2	6·0	6·0	3·0	45·0	45·0	30·0
	32 ...	0·2	6·0	4·0	2·0	30·0	120·0	60·0
	33 ...	0·3	8·0	4·0	4·0	60·0	120·0	60·0
	34 ...	0·4	6·0	3·0	1·5	30·0	120·0	90·0
	35 ...	0·4	24·0	16·0	6·0	60·0	120·0	120·0
	36 ...	0·6	24·0	6·0	1·5	90·0	180·0	120·0
Untreated controls	37 ...	0·2	0·2	0·2	<0·2	45·0	120·0	120·0
	38 ...	0·3	0·3	0·3	0·2	30·0	30·0	30·0
	40 ...	0·4	0·4	1·2	1·2	30·0	30·0	30·0
	43 ...	0·6	4·8	2·4	0·8	60·0	240·0	180·0
	X ...	0·8	0·4	0·4	0·4	—	—	—
	39 ...	0·8	0·8	0·6	0·4	30·0	120·0	120·0
	41 ...	0·8	0·6	0·6	1·6	15·0	60·0	60·0
	44 ...	0·8	0·8	1·6	6·4	15·0	45·0	45·0
	42 ...	1·2	1·6	1·6	0·8*	60·0	180·0	180·0

* Staphylococcus (toxin + cult.) injected into right udder of all sheep 16.12.38.

except in the early stages. An examination was made weekly and any secretion present was drawn off through the teat canal. The secretion was a watery fluid containing clots or inspissated pus.

From Table 3 and Chart 5, it will be seen that in the sheep which were given two doses of toxoid, the immediate antitoxin response tended to be more pronounced and the amount of antitoxin remained at a rather higher level than in those which received a single dose of toxoid. An occasional sheep however reacted well even to a single dose. Among the controls the amount of circulating antitoxin had slightly increased in five of the nine sheep during the four months the animals were kept before the test injection. After the test injection, the titres were on the whole higher on the 20th day than on the 10th or 31st day. Since in the titrations wide serum differences were used, the titres on the 20th and 31st days are often noted as the same, whereas by taking note of traces of hæmolysis in the titration tubes it was often apparent that the titre was slightly waning on the 31st day.

TABLE IV.

EXPERIMENT 4.—UNITS OF α ANTITOXIN PER C.C. SERUM.

RELATIONSHIP BETWEEN INITIAL TITRE AND THAT AFTER TOXOID AND/OR TEST DOSE.

Sheep.	*Average titre prior to test injection.*		*Average titre during 3 months after test injection.*	
37	0·2		95·0	
38	0·27	0·48	30·0	61·0
39	0·65		90·0	
40	0·8		30·0	
41	0·9		45·0	
42	1·3	1·6	140·0	95·0
43	2·1		160·0	
44	2·4		35·0	

	Sheep.	*Titre before toxoid.*		*Average titre during 3 months following toxoid injections.*		*Average titre during 3 months following test injection.*	
One dose Toxoid	25	0·2	0·3	2·1	3·2	25·0	35·0
	26	0·3		4·6		40·0	
	27	0·4		3·1		40	
	28	0·4	0·66	3·3	4·7	100·0	73·0
	29	0·8		4·0		50·0	
	30	0·8		7·0		70·0	
Two doses toxoid	31	<0·2	0·23 or less	5·0	4·7	40·0	63·0
	32	0·2		4·0		70·0	
	33	0·3		5·3		80·0	
	34	0·4	0·46	3·5	9·7	80·0	103·0
	35	0·4		15·3		100·0	
	36	0·6		10·5		130·0	

Table 4 also shows the relationship between the initial serum titre and that following the toxoid and/or test injections. It will be seen that after injecting toxoid the sheep with higher initial titres

respond by producing antitoxin more actively than do those with lower initial titres. Here the toxoid is acting as a secondary stimulus, the difference in titre being especially marked in the group receiving two doses of toxoid. Sheep with higher initial titres also respond more effectively to the subsequent test injection. Similarly in the control group the higher the average pre-injection level of antitoxin the more efficient is the staphylococcus + toxin test injection in producing antitoxin. Evidently in these instances the injection is acting as a more efficient secondary stimulus in sheep which have shown a satisfactory response to a primary stimulus. It can also be seen that the injection of toxin and culture may act as a far more potent immunising agent than even two doses of toxoid.

By comparing Chart 6, showing the relative sizes of the local reactions following the test injection, and Tables 3 and 4, giving the serum titre in the treated and control animals, the relationship between the two can be seen. In the control group there is a direct relationship. Thus the local reactions were largest in sheep 39, 37 and 43, animals which showed a high antitoxin response. In the toxoid groups, it is evident that those which had two doses of toxoid and in consequence higher average antitoxin titres were better protected in the sense that they gave on the whole smaller local reactions. After the two groups had been injected with staphylococcus + toxin, the antitoxin response tended to be better in the group which had previously received two doses of toxoid.

Thus in experiment 4 the controls showed more constitutional disturbance than those in the toxoid group and one of them had to be destroyed, while two other controls showed very severe local disturbance. Two doses of toxoid are more efficient than one and the effect of immunisation is still apparent after three months.

Experiment 5 (*March to May*, 1939). The object of this experiment was to compare the immunity response given by two doses of toxoid with that given by one or two doses of the same toxoid after precipitation with potash alum.

It was found necessary to examine blood samples from 110 ewes in order to find 30 with low antitoxin titres (1·2 units or less per c.c.). These 30 sheep were then divided into 6 groups and treated as follows:—

Group 1.—No. 65 to 69, unprecipitated toxoid, 2·5 and 5·0 c.c.

Group 2.—Nos. 45 to 49, alum toxoid, 2·5 and 5·0 c.c.

Group 3.—Nos. 55 to 59, alum toxoid, 0·5 and 1·0 c.c.

Group 4.—Nos. 50 to 54, alum toxoid, 5·0 c.c.

Group 5.—Nos 60 to 64, alum toxoid, 1·0 c.c.

Group 6.—Nos. 70 to 74, untreated controls.

Where two doses were given, the interval was 21 days, the alum toxoid in the meantime being preserved with 1:10,000 merthiolate. Sheep getting the single dose of alum toxoid were injected on the

same day as the other treated sheep were given their second dose. The alum toxoid caused the formation of a firm and rather persistent nodule, the size of which varied with the volume of toxoid injected. All 30 sheep were bled immediately before the first dose of toxoid was given to groups 1, 2 and 3, ten days after toxoid treatment was completed and finally 17 days after the test injection of staphylococcus. Ten days after toxoid treatment was completed, all 30 sheep, with two exceptions, were injected into one side of the udder with 8 c.c. toxin and 2 c.c. culture. The two exceptions were: No. 45, in which both teats were found to be blind so that the injection was given subcutaneously at the base of the teats, and No. 67 which lambed a few hours before it was due to be injected. The injection of No. 67 was therefore postponed for three days. All serum samples were titrated on the same day, using the Lh/50 toxin level for samples taken prior to treatment and the Lh/10 level for those taken subsequently. The titration results are shown in Table 5 and the sizes of the udder swellings in Chart 7.

The general and local reactions shown by the experimental sheep were as follows. Many of the sheep in groups 3, 4 and 6 showed inappetence during the first two days after the test inoculation. The controls showed much more severe local disease than the sheep in the protected groups, among which there were no pronounced differences as regards size. Two of the controls, Nos. 72 and 70, developed œdema which extended as far as the navel. Over this area a patch of skin measuring about 6 by 2 inches underwent necrosis and sloughed later together with a considerable amount of stinking inspissated pus. It should be noted that these were the two animals which had the lowest pre-injection titres. The three other control animals developed very large swellings from which considerable amounts of purulent secretion could be removed. Necrosis, with sloughing, involving the skin did not occur in any of the animals injected with toxoid, but one animal in each of groups 3 and 4 developed fluctuating swellings about an inch in diameter which on incision were found to contain inspissated pus. In sheep No. 67 in group 1 the injected udder half reached the size of a football and was doughy to the touch. The ewe was off her food and died two days later. On section the udder half was dark red in colour and microscopically, apart from intense congestion and hæmorrhage, masses of staphylococci could be seen in the acini. Sheep 56 (group 3) and 52 (group 4) lambed before the immunisation process was completed, and for this reason are not comparable with the remaining sheep in the experiment. Chart 7 and Table 5 show that No. 52 developed a very extensive mastitis and a high antitoxin concentration following the staphylococcus injection.

The titration results show, as in experiment 4, that the greater the amount of "natural antitoxin" present, the greater as a rule is the response to injections of toxoid. The same effect is seen in the control group after the test injection. In experiment 5 the antitoxin

Table V.

Experiment 5.—Units of α antitoxin per c.c. serum.

Sheep.		Serum of 23.3.39 (*before toxoid*)	Serum of 24.4.39 (*after toxoid*)	Serum of 10.5.39 (*after staph.*)
Alum toxoid (2·5 & 5·0 c.c.)	45	0·4	8·0	64·0
	46	0·8	12·0	96·0
	47	1·2	32·0	128·0
	48	1·6	24·0	96·0
	49	1·6	24·0	512·0
Alum toxoid 0·5 & 1·0 c.c.)	55	0·2	2·0	64·0
	56	0·6	8·0	16·0
	57	0·6	6·0	96·0
	58	0·8	6·0	32·0
	59	1·2	8·0	48·0
Toxoid (2·5 & 5·0 c.c.)	65	0·4	2·0	64·0
	66	0·8	12·0	128·0
	67	1·2	8·0	...
	68	1·6	8·0	16·0
	69	1·6	16·0*	24·0
Alum toxoid (5·0 c.c.)	50	0·4	3·0	48·0
	51	0·8	16·0	16·0
	52	0·8	12·0	256·0
	53	1·2	32·0	48·0
	54	1·6	24·0	96·0
Alum toxoid (1·0 c.c.)	60	0·4	4·0	12·0
	61	0·8	4·0	128·0
	62	1·6	24·0	24·0
	63	1·6	12·0	512·0
	64	6·4	24·0	24·0
Untreated controls	70	0·6	0·4	12·0
	71	0·8	3·2	24·0
	72	0·8	0·8	48·0
	73	1·6	1·6	64·0
	74	1·6	6·4*	64·0

Toxoid injections = Two doses on 24.3.39 and 14.4.39.
One dose on 14.4.39.
*Staphylococcus (toxin + cult.) injected into right udder of all sheep 24.4.39.

titres in the control sheep were low by comparison with those in experiment 4, and in general, except with sheep No. 52, any direct relationship between the size of the mastitis and the antitoxin response is not so evident.

Judging by the average antitoxin titres, there is little to choose between the protection afforded by alum toxoid given in two doses of 2·5 and 5·0 c.c. and a single dose of 5·0 c.c., while the protection afforded by two doses (2·5 and 5·0 c.c.) of the unprecipitated toxoid and by the smaller amounts of alum toxoid used is of a lower order.

Discussion.

Burnet (1929) and a number of other workers have shown that rabbits actively or passively immunised against staphylococcus toxin are more resistant than normal animals to injections of living staphylococci. The more recent experiments of Downie (1937) have demonstrated that rabbits actively immunised with staphylococcus toxin or toxoid acquire considerable resistance to intracutaneous injections of living staphylococci or toxin. It was likely, therefore, that sheep immunised with toxoid, as in the present experiments, would suffer less severely than untreated sheep from the effects of living staphylococci and toxin given intramammarily. Nevertheless, it must be remarked that the conditions under which the present experiments were performed differ in several respects from those that would obtain in nature. Presumably, under natural conditions, many of the animals being immunised would be pregnant and the mammary tissue preparing to function. It is also possible that, if the present experiments had been done on sheep exposed to adverse nutritional and climatic influences, the test inoculations might have caused more severe general and local effects. As it was, under the relatively favourable conditions in which the sheep were kept, out 25 control sheep, three died or had to be destroyed following the udder injections. With one exception, all the experimental sheep were in the dry period ; in the exceptional case, the test was made on a ewe which had lambed three days previously and the effects were so severe that the animal quickly died in spite of toxoid treatment.

Throughout this work the necessity was realised of selecting sheep with only small amounts of natural antitoxin in the blood, in order that the results might be as nearly comparable as possible. In the same connection, as many animals as could be conveniently accommodated were included in each group. The selection of suitable animals was not really a simple matter since only one blood examination could be made prior to purchase, and in some cases it was noted that the antitoxin titre tended to rise before the animals were actually brought under experiment.

Mastitis in sheep is no doubt a good deal commoner than might be imagined from the relatively few references in the literature ; many cases are not brought to the notice of the veterinarian and

many others must pass unrecorded. Even so, the present experiments were carried out not so much with a view to investigating the prophylaxis of the ovine disease as to testing the extent to which specific toxoid treatment might modify the course of acute staphylococcus mastitis in any animal species, and in order to gain experience of the kind of toxoid that should be used and the antitoxin response likely to be aroused. As part of this investigation, a further experiment with alum toxoid would have been advantageous but this could not be undertaken. Obviously, however, the final answer must await a field trial in flocks where the natural disease is prone to occur (*e.g.*, in Cyprus), preferably using an alum toxoid in single dose or in two repeated doses. In the meantime, it may be concluded that, although immunity against staphylococci is not wholly an antitoxin effect, immunisation with toxoid in staphylococcus mastitis should be at least as efficient as the vaccines of Bridré and others which are stated to have given favourable results in practice.

Summary.

1. The opportunity has been taken to bring together the literature references on ovine mastitis, more particularly in respect of the staphylococcus form of the disease.

2. Treatment with specific toxoid prevents the general effects and to a considerable extent the local effects resulting from an infusion of staphylococcus culture and toxin into the udder. It can probably be assumed that toxoid would act similarly in nature.

3. Under the conditions of the experiments, two injections of toxoid at an interval of about three weeks gave a better immunity than a single dose. Immunity was evident for at least three months after the second injection. In a single experiment one dose of alum toxoid gave a rather better immunity response than two doses of unprecipitated toxoid.

4. The amount of antitoxin developing in sheep treated with toxoid tended to be greater in animals with higher pre-injection titres. In sheep judged to be adequately protected antitoxin levels averaging 8 to 24 units per c.c. serum were observed 10 to 20 days after treatment, representing increases of 6 to 15 times the pre-injection titre.

5. Unprotected sheep with low antitoxin titres suffer more severely from the test inoculation than those with higher titres.

Acknowledgements.

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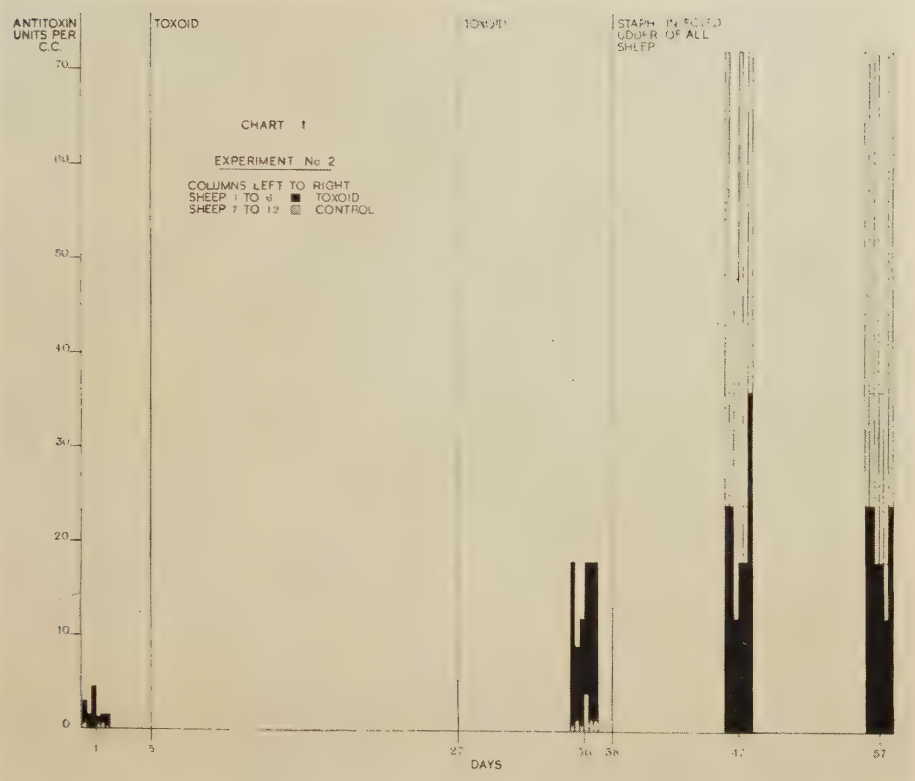
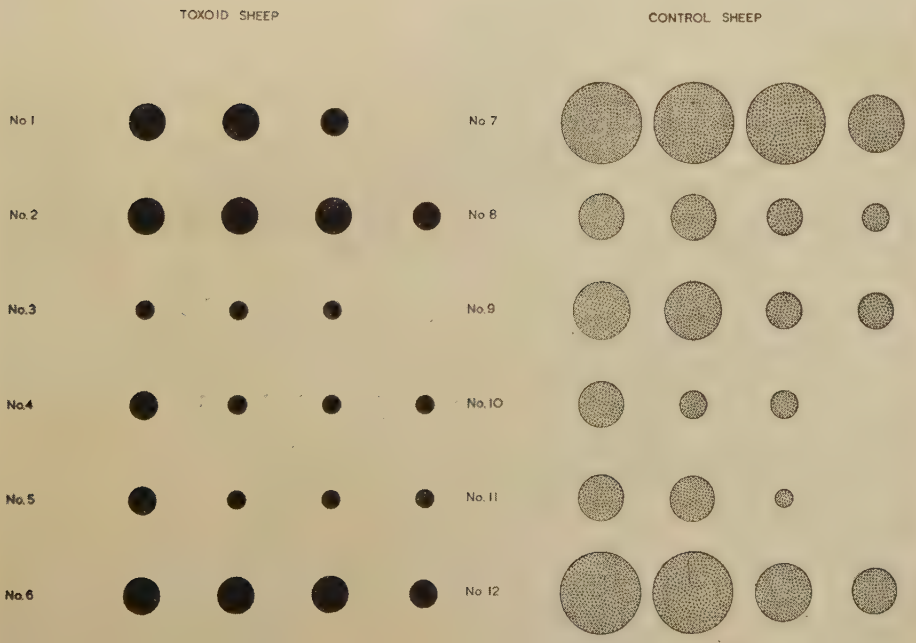


CHART 2.

EXPERIMENT No. 2



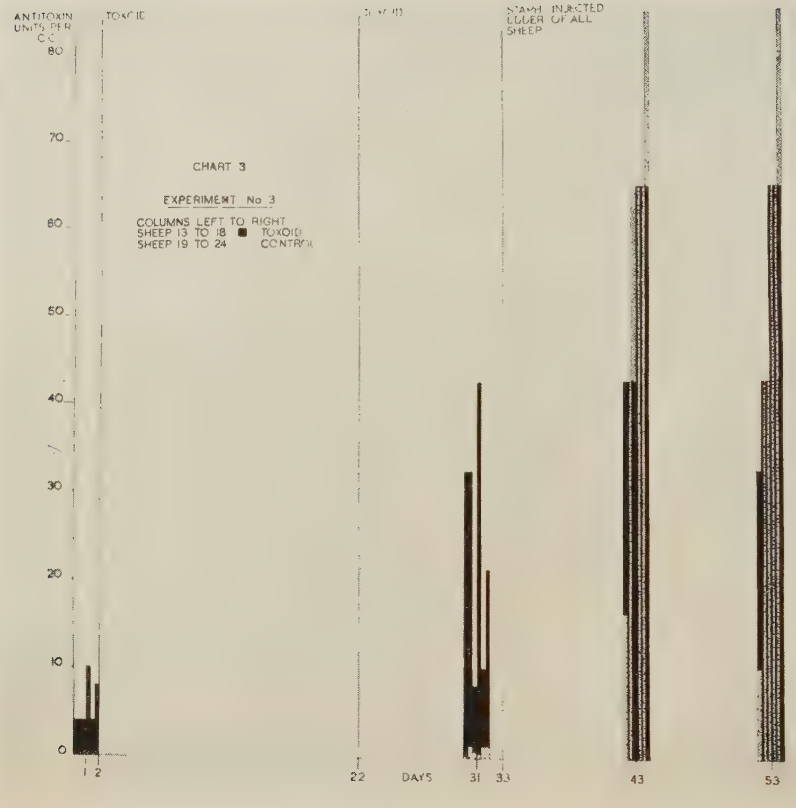
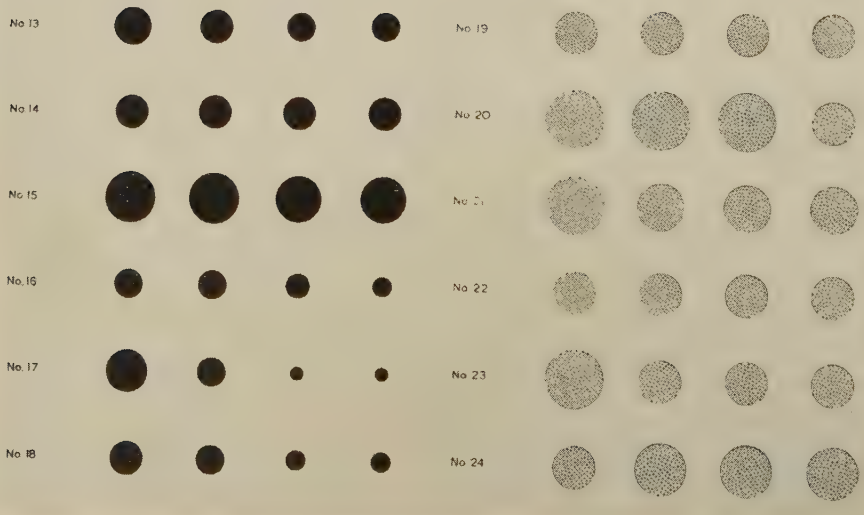


CHART 4

EXPERIMENT No. 3

TOXOID SHEEP

CONTROL SHEEP



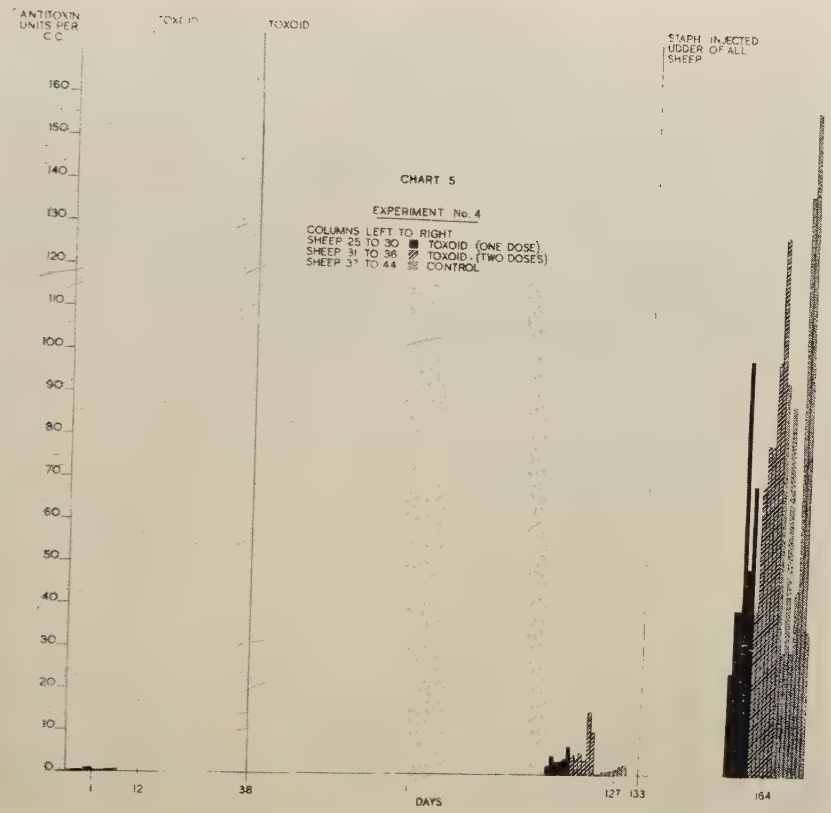

ANTITOXIN UNITS PER C.C.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
TOXOID
STAPH. INJECTED UDDER OF ALL SHEEP
CHART 5
EXPERIMENT No. 4
COLUMNS LEFT TO RIGHT
SHEEP 25 TO 30 TOXOID (ONE DOSE)
SHEEP 31 TO 36 TOXOID (TWO DOSES)
SHEEP 37 TO 44 CONTROL
1
12
38
127
133
164
DAYS

CHART 6

EXPERIMENT No. 4

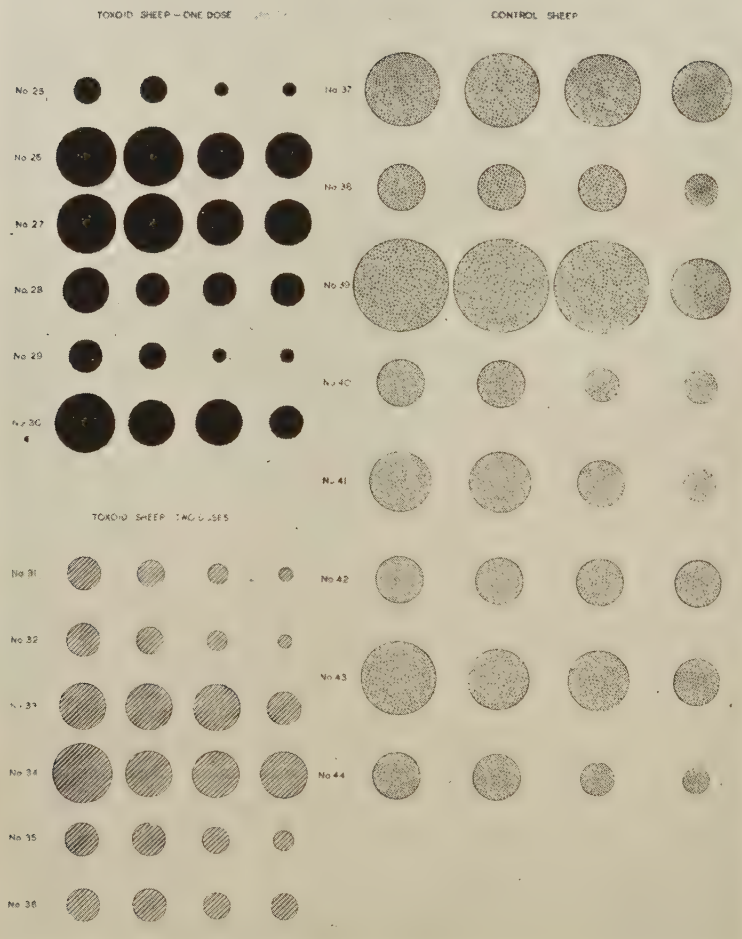
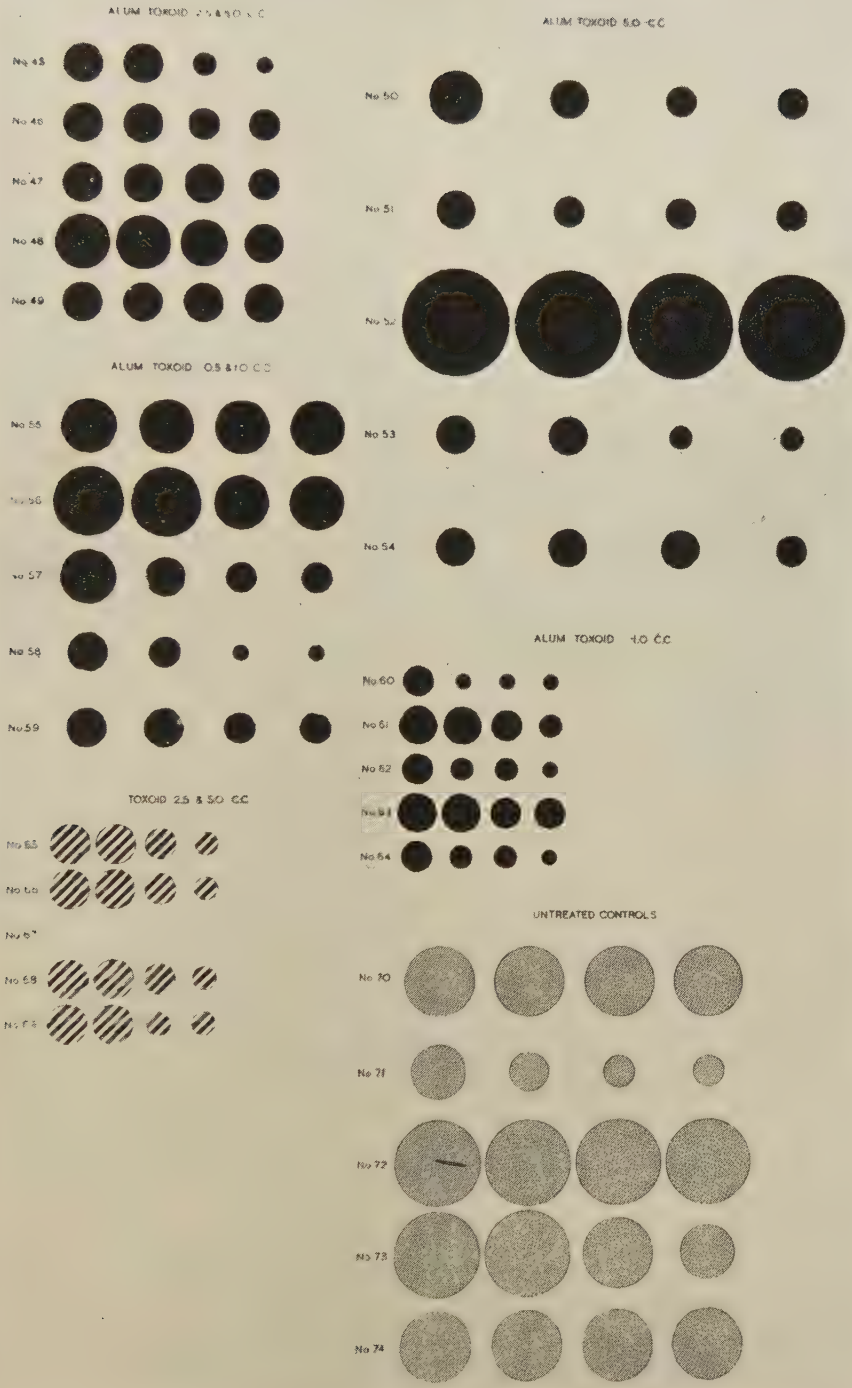


CHART 7

EXPERIMENT No 6



SERUM PROTEIN CHANGES IN DIFFERENT TYPES OF CATTLE DURING IMMUNISATION AGAINST RINDERPEST VIRUS

By M. H. French, M.A., PH.D.

In previous publications[1,2] the changes in the serum protein fractions of ox blood during infection with, and during immunisation and hyperimmunisation against, rinderpest virus have been described. A criticism raised against this work was that in Tanganyika the zebu cattle possess a certain natural resistance to this disease and possibly, therefore, the serum protein changes described might not be the same as those occurring in more highly susceptible cattle. The object of this further study is to determine how far the changes resulting from active immunisation against rinderpest vary between different breeds of cattle.

For this study two shorthorned zebu cattle of the type used in the previous investigations, and which form by far the greater proportion of the cattle in Tanganyika Territory, have been compared with two animals of each of the following breeds: ¾ grade Ayrshires, ¾ grade Friesians, and the other indigenous type known as the Ankole (Sanga) type. The Ankoles are very different in conformation from the zebu and are highly susceptible to rinderpest. In the routine field inoculations against this disease the hyperimmunised serum of moderate titre, prepared by the intra-rumenal hyperimmunisation of zebu cattle, has not proved successful for the protection of these more highly susceptible Ankoles. A serum of high protective value is required for them. Severe rinderpest reactions also occur in the higher graded European types of cattle and they are generally considered not to have acquired the enhanced resistance attributed to the zebus.

The general plan of this investigation has been the same as in the previous studies. The animals were inoculated simultaneously with citrated virulent blood and with protective serum on opposite sides of the body. Blood was taken before inoculation with virus and serum, and then at intervals during the course of the subsequent reaction. After the blood had clotted it was centrifuged to remove all the clot and red cells, and then the clear serum was removed and analysed. The various protein fractions were separated by means of Howe's technique[3] and the nitrogen estimations were made by means of a micro-Kjeldahl method.

The animals examined were all yearlings of approximately the same age. During the period they were confined for the immunisation they all received the same ration of hay and concentrates, and the rate of feeding was such that under normal conditions they would have gained weight steadily.

One animal of each breed was inoculated with serum and virus, and the second animal of each pair was done a fortnight later with a different serum, and virus from another animal. Actually, for the first inoculations the virus givers had shown very indefinite reactions and the four experimental animals failed to react. They were given a further inoculation of serum and goat virus a week later and to this they reacted. In the second series the animals all reacted to the virus inoculation (in this case cattle virus). In the first series the Ankole calf received Ankole immune serum whilst the others were given normal hyperimmune zebu serum. In the second series all the animals were inoculated with Ankole immune serum.

The Details of the Individual Cases and the Results Obtained.

Zebu Ox, 4824.—This ox was given 10 ml. citrated blood from the doubtful reactors containing tartar emetic and trypan blue to guard against the accidental introduction of trypanosomes and piroplasms. Normal routine hyperimmune zebu serum was simultaneously inoculated (80 ml.). This large dose was given to bring this ox into line with the following animals which were given the large dose of serum to avoid any risks of them dying from the subsequent reactions.

As a definite thermal reaction did not develop the animal was given 5 ml. citrated virulent blood from a goat a week later, together with a further 40 ml. serum.

A mild and slightly delayed thermal reaction followed and the animal remained in good condition. The appetite was not noticeably diminished and no diarrhoea developed though there was a slight discharge from the eyes and nose.

Table I shows the serum protein for this ox.

Table I.

Serum Protein Changes during Immunisation of Zebu Ox, No. 4824.

	17.1.38	3.2.38	9.2.38	15.2.38
Total N per cent.	0·8610	1·2040	1·1270	1·0360
Non-Protein N per cent. ...	0·0388	0·0506	0·0388	0·0294
Total Protein N per cent. ...	0·8222	1·1534	1·0882	1·0066
Euglobulin N per cent.	0·0490	0·1890	0·1929	0·1722
Pseudoglobulin N per cent. ...	0·3654	0·4872	0·4060	0·3654
Total Globulin N per cent. ...	0·4144	0·6762	0·5989	0·5376
Albumin N per cent.	0·4078	0·4772	0·4893	0·4690
Euglobulin as percentage of Total Protein	5·96	16·39	17·73	17·11
Pseudoglobulin as percentage of Total Protein	44·44	42·24	37·31	36·30
Albumin as percentage of Total Protein	49·60	41·37	44·96	46·59

These changes can be summarised as an initial rise in the total protein and total globulin contents followed by a slower decline.

The euglobulin fraction showed the greatest proportional changes; this fraction increased in value until about the third week and then fell slightly. The pseudoglobulin and albumin fractions were decreased as the euglobulin proportions increased. This is an absolutely typical picture and agrees with the majority of cases reported upon in the earlier publication[1].

Three-quarter Grade Ayrshire Heifer, 318.—This animal received identical treatment, virulent blood and serum, as the above zebu ox. The subsequent thermal and clinical reactions were almost identical with those described for the zebu. The animal continued to look well and was not noticeably off feed.

Table II presents the analytical data for this heifer.

TABLE II.

SERUM PROTEIN CHANGES DURING IMMUNISATION OF ¾ GRADE AYRSHIRE HEIFER, NO. 318.

	17.1.38	3.2.38	9.2.38	16.2.38
Total N per cent.	0·9170	0·9690	1·1270	1·0220
Non-Protein N per cent. ...	0·0506	0·0294	0·0294	0·0294
Total Protein N per cent. ...	0·8664	0·9396	1·0976	0·9926
Euglobulin N per cent.	0·1050	0·1570	0·2126	0·1885
Pseudoglobulin N per cent. ...	0·3015	0·3454	0·3760	0·3466
Total Globulin N per cent. ...	0·4065	0·5024	0·5886	0·5351
Albumin N per cent.	0·4599	0·4372	0·5090	0·4575
Euglobulin as percentage of Total Protein	12·12	16·71	18·46	18·99
Pseudoglobulin as percentage of Total Protein	34·80	36·76	34·26	34·92
Albumin as percentage of Total Protein	53·08	46·53	46·38	46·09

The total protein and total globulin again showed the initial rise and subsequent decrease though the maxima were reached a little later. The euglobulin proportions again increased to a slightly higher level than recorded for the zebu ox and had not begun to decrease by the third week. The pseudoglobulin proportions remained roughly constant, but the albumin percentage decreased.

Again the serum protein changes agree with those previously published, both in the nature and the extent of the variations.

Three-quarter Grade Friesian Ox, 4849.—The treatment of this ox was identical with those of the two previously described cases. Again the subsequent mild thermal and clinical reactions were as described above, and the animal looked well at the time he was discharged. It is probable that this animal had been vaccinated against rinderpest some months before immunisation.

Table III summarises the serum protein variations for this ox.

TABLE III.

SERUM PROTEIN CHANGES DURING IMMUNISATION OF ¾ GRADE FRIESIAN OX, No. 4849.

	17.1.38	3.2.38	9.2.38	15.2.38
Total N per cent.	1·0020	1·0421	1·1180	1·0360
Non-Protein N per cent. ...	0·0340	0·0421	0·0388	0·0294
Total Protein N per cent. ...	0·9680	1·0000	1·0792	1·0066
Euglobulin N per cent.	0·1085	0·1091	0·1827	0·1722
Pseudoglobulin N per cent. ...	0·3248	0·3469	0·4075	0·3654
Total Globulin N per cent. ...	0·4333	0·4560	0·8902	0·5376
Albumin N per cent.	0·5347	0·5440	0·4890	0·4690
Euglobulin as percentage of Total Protein	11·21	10·91	16·93	17·11
Pseudoglobulin as percentage of Total Protein	33·55	34·69	37·76	36·30
Albumin as percentage of Total Protein	55·24	54·40	45·31	46·59

The total amounts of globulin and protein in the serum varied in the normal manner. The changes in the euglobulin were slightly different in that no increase took place until after the first week following innoculation, and the increase in the proportional amounts continued until the third week, though the increase from the second to the third week was very small. The pseudoglobulin percentages increased slightly, but the albumin proportions decreased.

These changes are somewhat different from those above but still agree with certain animals recorded in the previous study, except that the point of the maximum euglobulin proportions has been somewhat delayed. This delay may be due to the probable previous vaccination in combination with the large dose of protective serum.

Ankole Ox, 4850. This animal was treated as the three previous ones except that immune serum made from another Ankole animal was used to protect against the virus. The doses given were the same as in the above cases.

The subsequent reaction was very similar in nature to those above, the thermal reaction was mild and delayed and the clinical symptoms were confined to a slight lachrymation. No diarrhoea developed and the animal continued to eat and look well.

The serum protein changes are shown in Table IV.

Again the total protein and total globulin changes followed the usual course. The euglobulin proportional amounts showed no change at the end of the first week but then increased to a maximum value in the third week. The pseudoglobulin proportions did not vary appreciably but the albumin percentages fell to the time of maximum euglobulin development.

These changes are normal except for a time lag of one week in reaching the point of maximum euglobulin content. The level

of euglobulin reached, though slightly higher than for the three previous cases, is not higher than that recorded for certain zebu oxen in the previous study.

TABLE IV.

SERUM PROTEIN CHANGES DURING IMMUNISATION OF ANKOLE OX, No. 4850.

	17.1.38	3.2.38	9.2.38	15.2.38
Total N per cent.	1·0080	1·0780	1·1900	1·0150
Non-Protein N per cent. ...	0·0420	0·0388	0·0294	0·0294
Total Protein N per cent. ...	0·9660	1·0392	1·1606	0·9856
Euglobulin N per cent.	0·1264	0·1442	0·1750	0·2030
Pseudoglobulin N per cent. ...	0·3329	0·3466	0·4075	0·3272
Total Globulin N per cent. ...	0·4593	0·5908	0·5825	0·5302
Albumin N per cent.	0·5067	0·5484	0·5781	0·4554
Euglobulin as percentage of Total Protein	13·09	13·88	15·08	20·60
Pseudoglobulin as percentage of Total Protein	34·46	33·35	35·11	33·20
Albumin as percentage of Total Protein	52·49	52·77	49·81	46·20

With the second series of immunisations no complications arose because of uncertain virus. The following animals all reacted to their first inoculation of virulent blood.

Zebu Ox, 4858.—This ox was given a large dose (80 ml.) of Ankole serum and underwent a mild clinical but definite thermal reaction. There was no delay in the onset of fever, no marked loss of appetite occurred and no diarrhoea developed.

The serum protein changes are set out in Table V.

TABLE V.

SERUM PROTEIN CHANGES DURING IMMUNISATION OF ZEBU OX, No. 4858.

	2.2.38	9.2.38	14.2.38	21.2.38
Total N per cent.	1·1270	1·2110	1·0220	1·0220
Non-Protein N per cent. ...	0·0396	0·0500	0·0406	0·0388
Total Protein N per cent. ...	1·0874	1·1610	0·9814	0·9832
Euglobulin N per cent.	0·0932	0·1554	0·1879	0·1806
Pseudoglobulin N per cent. ...	0·4045	0·4090	0·3657	0·3760
Total Globulin N per cent. ...	0·4977	0·5644	0·5536	0·5566
Albumin N per cent.	0·5897	0·5966	0·4278	0·4266
Euglobulin as percentage of Total Protein	8·57	13·38	19·14	18·37
Pseudoglobulin as percentage of Total Protein	37·20	35·23	37·26	38·23
Albumin as per cent. of Total Protein	54·23	51·39	43·60	43·39

The changes in total protein and total globulin followed the usual course and the euglobulin proportions reached their maximum

value about the end of the second week. The subsequent small decline in the euglobulin percentage by the end of the third week was again recorded. The pseudoglobulin proportions remained at about the same level throughout and did not decrease as in the case of the previous zebu ox. The albumin percentages showed the usual fall in value.

This case is again typical of others reported upon in the previous publication.

Three-quarter Grade Ayrshire Heifer, 313.—This animal was also protected by means of 80 ml. Ankole serum, and there was no delay in the subsequent reaction. The animal was discharged in good condition after a mild clinical but definite thermal reaction which was almost identical with that of the above zebu ox, 4858.

The serum protein charges are presented in Table VI.

Table VI.

Serum Protein Changes during Immunisation of ¾ Grade Heifer, No. 313.

	2.2.38	9.2.38	14.2.38	21.2.38
Total N per cent.	0·9994	1·0129	1·1046	1·0657
Non-Protein N per cent. ...	0·0350	0·0388	0·0350	0·0322
Total Protein N per cent. ...	0·9644	0·9741	1·0696	1·0335
Euglobulin N per cent.	0·0952	0·0979	0·2006	0·1941
Pseudoglobulin N per cent. ...	0·3395	0·3409	0·3671	0·3523
Total Globulin N per cent. ...	0·4347	0·4388	0·5677	0·5464
Albumin N per cent.	0·5297	0·5353	0·5019	0·4871
Euglobulin as percentage of Total Protein	9·87	10·05	18·76	18·78
Pseudoglobulin as percentage of Total Protein	35·21	35·00	34·32	34·09
Albumin as percentage of Total Protein	54·92	54·95	46·92	47·13

In this case there was very little increase in the proportions of euglobulin at the end of the first week but then a more marked rise occurred. The level reached at the end of the second week was that usually found in zebu cattle, and this value was maintained unaltered until the end of the third week. The pseudoglobulin percentages showed a slight tendency to decrease, whilst the albumin proportions decreased.

Three-quarter Grade Friesian Ox, 4856.—This animal was treated identically with the two previous animals and received 80 ml. Ankole serum. He remained in good condition and was not noticeably off feed. This animal had probably been vaccinated against rinderpest before he came into this experiment. He had a mild clinical and a definite though delayed thermal reaction. The height of the thermal response was not reached until the twelfth day and the temperature remained almost constant until the ninth day.

The serum protein changes are shown in Table VII.

TABLE VII.
SERUM PROTEIN CHANGES DURING IMMUNISATION OF ¾ GRADE FRIESIAN OX, No. 4856.

	2.2.38	9.2.38	16.2.38	23.2.38
Total N per cent.	0·8680	1·0059	1·0080	1·0360
Non-Protein N per cent. ...	0·0381	0·0419	0·0506	0·0294
Total Protein N per cent. ...	0·8299	0·9640	0·9574	1·0066
Euglobulin N per cent.	0·0560	0·0615	0·1742	0·1858
Pseudoglobulin N per cent. ...	0·3039	0·3546	0·3654	0·3636
Total Globulin N per cent. ...	0·3599	0·4161	0·5396	0·5494
Albumin N per cent.	0·4700	0·5479	0·4178	0·4572
Euglobulin as percentage of Total Protein	6·75	6·38	18·19	18·46
Pseudoglobulin as percentage of Total Protein	36·62	36·78	38·17	36·12
Albumin as percentage of Total Protein	56·63	56·84	43·64	45·42

The euglobulin percentage remained unaltered at the end of the first week as would be anticipated from the fact that the reaction was delayed. The proportions of this fraction then increased and no fall in value had occurred by the end of the third week when the ox was discharged. The pseudoglobulin proportions remained almost constant but the albumin fraction decreased after the end of the first week.

These changes are normal if allowance is made for the delayed reaction.

Ankole Heifer, 4854.—This animal was treated exactly as in the three previous cases, and there was no delay in the incidence of the subsequent fever. A high thermal reaction followed and marked clinical symptoms were observed. By the tenth day this heifer was off its food, and on the thirteenth day diarrhoea had developed with much straining and the passage of bloody mucus. Discharges were also observed from the eyes and nose. The animal then began feeding again and was discharged at the end of the third week.

The data on the serum protein changes is given in Table VIII.

TABLE VIII.
SERUM PROTEIN CHANGES DURING IMMUNISATION OF ANKOLE HEIFER, No. 4854.

	2.2.38	8.2.38	14.2.38	21.2.38
Total N per cent.	0·9520	1·0880	1·0780	1·0640
Non-Protein N per cent. ...	0·0488	0·0480	0·0488	0·0402
Total Protein N per cent. ...	0·9032	1·0400	1·0292	1·0238
Euglobulin N per cent.	0·0791	0·0700	0·1036	0·2180
Pseudoglobulin N per cent. ...	0·3045	0·3674	0·3654	0·3812
Total Globulin N per cent. ...	0·3836	0·4374	0·4690	0·5992
Albumin N per cent.	0·5196	0·6026	0·5602	0·4246
Euglobulin as percentage of Total Protein	8·76	6·73	10·07	21·29
Pseudoglobulin as percentage of Total Protein	33·71	35·33	35·50	37·24
Albumin as percentage of Total Protein	57·53	57·94	54·43	41·47

The euglobulin proportions showed a decrease at the end of the first week but had risen again to slightly above the initial value after two weeks. A marked rise then took place to the usual high level at the end of the third week. The pseudoglobulin proportions increased slightly from the beginning. The albumin percentages showed an increase in value at the end of the first week and then decreased.

The changes in this heifer are not typical of those during immunisation but resemble more closely those occurring during an attack of rinderpest, from which the animal recovered spontaneously. This is probably to be explained by the greater severity of the reaction in this beast.

Review of the Above Cases.

To make the above results more clear it is advisable to tabulate them in the following manner before proceeding to their general discussion.

Table IX.

Review of the Changes in the Proportional Amounts of the Serum Protein Fractions.

Animal.	*Breed.*	*Euglobulin per cent.*	*Pseudoglobulin per cent.*	*Albumin per cent.*
4824	Zebu	Increased to end of 2nd week	Decreased with Euglobulin increase	Decreased
4858	„	„	Remained constant	„
318	¾ grade Ayrshire	Increased end of 3rd week	Remained constant	„
313	„	Unaltered after 1st week then increased to end of 3rd week	Slight decrease with Euglobulin increase	„
4849	¾ grade Friesian	Unaltered after 1st week then increased to end of 3rd week	Slight increase	„
4856	„	„	Remained constant	„
4850	Ankole	Unaltered after 1st week then increased to end of 3rd week	Remained constant	„
4854	„	Decreased at end of 1st week then increased to end of 3rd week	Slight increase	„

Discussion.

Confirmation has been obtained of previous findings that marked changes take place in the serum protein fractions during immunisation against rinderpest. Further, the changes are of the same type and magnitude as previously recorded and occur in all four types of cattle investigated. These changes consist of an increase

in the globulin proportions and a decrease of those of the albumin fraction. The euglobulin fraction is more affected than the pseudoglobulin, though the latter can increase.

In a previous publication it was suggested that the rinderpest antibodies are possibly associated with the more soluble portion of the euglobulin fraction and to a smaller extent with the least soluble portions of the pseudoglobulin fraction. If this is so, then with present methods of separation (by varying the salt concentrations) it is easy to see that slight changes in the reactions of the solutions may cause an increased antibody content to be shown by increases in either of these two fractions, though the euglobulin fraction will usually show the greater change.

The present results, with different types of cattle, agree with this hypothesis. The fact that in many of the non-zebu animals there was a lag in reaching the maximum globulin content does not affect the main conclusions that immunisation against rinderpest is attended by an increase in the globulin fraction, which is due chiefly to an increase in the proportions of euglobulin, though the pseudoglobulin proportions may occasionally be slightly increased.

The fact that the present results were obtained with different types of cattle, which included the highly susceptible Ankoles, shows that the original observations on zebus, which may have an enhanced resistance to rinderpest, are entirely valid.

Conclusions.

(1) The results obtained with grade Ayrshires and Friesians, and also with highly susceptible Ankoles, show that immunisation against rinderpest is attended by changes in the serum protein fractions which are not dependent on breed but are a direct result of immunisation.

(2) The changes consist of an increase in the globulin and a decrease of the albumin proportions.

(3) The globulin increase is due chiefly to an increase of the euglobulin fraction, though occasionally the pseudogloblin is also increased.

(4) The time taken to develop the maximum euglobulin content appears shorter with zebu than with the other types of cattle examined, but the maximum value reached is of the same order for each type.

Ackowledgments.

I am indebted to the Director of Veterinary Services for permission to publish this article, and also to Mr. S. A. Evans, M.R.C.V.S., for the clinical observations on the experimental animals.

References.

[1]French, M. H. (1936). *J. Comp. Path. Ther.* **49**. 118.
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ON VACCINATION OF CALVES AGAINST INFECTIOUS ABORTION

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In the beginning of this century B. Bang (collaborating with Stribolt) carried out the encouraging experiments* on vaccination against infectious abortion in cattle, which gradually led veterinarians in many countries to employ extensively immunisation of cattle by subcutaneous injection of living non-attenuated abortus bacilli on non-pregnant cows and heifers.

As is well known, in the last decade several countries have prohibited this employment of living bacterial cultures. This applies, for example, to two of our neighbouring countries, Germany and Norway. This prohibition has been made because of the uncertainty as to the effect of the vaccination, the production of additional spreaders of milk-infection (resulting in increased danger of infection to man), and maintenance of the infection in the vaccinated stocks—with the result that the ideal, a rational eradication of the disease, was set aside.

Concurrent with this prohibition of vaccination there has been in the same countries, as well as in others, an increasing interest in a rational (complete) eradication of infectious abortion through blood examination, isolation and disinfection. Among the countries leading in this respect, Norway has to be mentioned as the foremost, where the disease indeed has been combated most energetically and admirably, especially in the last four to five years, so that now it is almost eradicated (Holth and Thorshaug)†. Also in the United States of America, Germany, Denmark and several other countries blood examination of the stocks is now being employed extensively and increasingly, and appropriate measures are adopted accordingly with, as a rule, results that may be said to be very promising. Nevertheless, Götze‡, as well as Karsten and Ehrlich§ (Germany), are probably rather too optimistic when they think it will be practicable within a few years to eradicate this disease entirely in Germany. Thus Götze says: "Ich habe die feste Ueberzeugung gewonnen, dass die Tilgung der Rinderbruzellose durch das Absonderungs—und Ausmerzverfahren bei Aufbietung und gleicher Ausrichtung aller Kräfte in 3-4 Jahren im gesamten Reichsgebiet möglich ist."

On the whole, however, the reports from several countries show plainly that stocks can be rid of this disease at a reasonable and

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* Published by O. Bang (1911) in Klimmer and Wolff-Eisner's "Handbuch der Serumtherapie . . .", Leipzig, **2**, 211.

† *Norsk Veterinaer-Tidsskrift.* (1938). **50**, 373.

‡ *Berliner Tierärztliche Wochenschrift.* (1938). p. 141.

§ *Ibid.* (1938). p. 274.

affordable cost as long as the disease is present only in a latent form —which is very often the case. The same applies to most of the stocks in which abortion occurs now and then in the chronic form. In fact it is only in a minority of the infected stocks (amounting to 12·7 per cent. on the island of Bornholm,* where all the herds, about 3,800, comprising about 39,000 mature animals are serologically examined) that it will be practically impossible to accomplish eradication of the disease in this way. There are especially large stocks in which the infection is acute and outbreaks come in rapid succession.

In the last-mentioned category of stocks, at least for the present, we have to proceed in other ways in combating the disease; it is in these stocks that vaccination seems to be required as a temporary adjuvant for reduction in the number of abortions. Only when the incidence of infectious abortion has been markedly decreased (or when it has been relatively slight, even from the start) within a certain district or in the whole country will it seem absolutely necessary to prohibit vaccination with living cultures.

From the literature it appears as if most authors agree that the frequency of abortion may be decreased simply by subcutaneous injection of a living bacterial culture into the cows a couple of months before they are put to the bull. But with this their agreement ceases. Opinions about the numerical results obtained in this way differ greatly. It would take us, however, too far here to go into details concerning this question in other countries, and I shall limit myself here to the Danish investigations in this field.

Extensive experiments, with controls, carried out through several years (1913 to 1919) by the State Veterinary Serum Laboratory† did not lead to satisfactory results with the method employed. In a material of altogether 871 heifers, half of which were vaccinated while the other half served as controls, the percentage of abortion was reduced only from 36·8 in the controls to 23·7 in the vaccinated animals. On the basis of these experiments the Serum Laboratory has hitherto considered it inexpedient to furnish living cultures as abortion vaccine.

O. Bang* thought he obtained better results in his vaccination experiments, although they included no proper controls. In his material the percentage of abortion appeared to be reduced from 41·0 to 16·7 and in the heifers from 54·6 to 16·2.

Nothwithstanding many efforts in various ways, since the publication of B. Bang's experiments from 1902-1910 nothing of practical value has really been published concerning vaccination of calves against infectious abortion till 1930, when J. M. Buck† presented a most interesting communication from the Bureau of Animal

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* cf. Axel Thomsen. (1938). *Acta Pathol. et Microbiol. Scand. Suppl.*, **37**, 532.

† C. O. Jensen. (1921). Beretning fra Det andet nordiske Veterinaermøde i Stockholm, p. 82.

* *Medlemsblad for Den danske Dyrlaegeforening.* (1927). **10**, 410.

† *Journal of Agricultural Research.* (1930). **41**, 667.

Industry, U.S.A.—a country which, on the whole, may be said for several years to have been the leading authority in Brucella research.

In this paper, Buck gave a detailed account of some laboratory experiments concerning vaccination of *calves* against infectious abortion, involving subcutaneous injection of living cultures of different virulence. The idea of these experiments was that by vaccinating the animals as calves it might perhaps be possible to obtain the advantage that the infection produced in the animals by the injection of the culture will be more apt to pass in the long period before the first pregnancy—perhaps particularly through the circumstance that the affinity of the abortus bacilli for the quite undeveloped udder in the calves might be less pronounced than the affinity in the udder of heifers and cows.

Buck's first experiment comprised 18 calves, from five to eight months old, twelve of which were vaccinated with a *single* subcutaneous injection of living abortus bacilli (20 c.c. of a culture), while six served as controls. The animals stood this treatment well; for two to three days there was some fever, together with lassitude and loss of appetite, but no abscess appeared. Two of the calves (one treated and one control) had to be excluded from the results for particular reasons. The animals were bulled nine months after the vaccination. The remaining 16 animals were infected *per os* about five months after service. The result of the experiment was very promising, as all the eleven vaccinated animals later calved normally, and gave birth to living calves. Still, by inoculation of guinea-pigs the presence of *Br. abortus* bacilli was demonstrated in the placenta, plus colostrum in one animal, and in the colostrum alone in another. Of the five controls three aborted, and abortus bacilli were demonstrated in all three cases in the foetus and in colostrum; two calved normally, with no abortus bacilli in the placenta or colostrum.

In the following pregnancy there was also observed a corresponding strong immunity in the same treated animals (with new controls in this experiment).

In no instance did the injection of the living abortus bacilli produce even a mere suggestion of sterility in the treated animals, and the bacilli of the vaccine had only a slight tendency to localise in the udder; furthermore, the vaccinated animals were as a rule resistant to a subsequent infection of the udder. As a rule, the often strong serum reactions induced through vaccination subsided markedly in a few (three to four) months—in striking contrast to the findings in adult animals. As a remnant of antibody, however, there often remained a suggestion in the form of capacity for agglutination in dilution 1 : 25 or 1 : 50.

In the following year Buck continued his experiments on veccination of calves, in collaboration with W. E. Cotton and H. E. Smith, and in 1934 a new report was published,* concerning 33 calves, from

* *Journal of the American Veterinary Medical Association.* (1934). Vol. 85 ; N.S. 38, 389.

four to six months old, 17 of which were vaccinated exactly as in the preceding experiment, while 16 were used for controls. The dose of vaccine was 10 c.c. (5 c.c. injected on each side of the neck) of a bacterial suspension 10 times as concentrated as Tube 1 in McFarland's nephelometer. While this experiment was largely a reproduction of the preceding, it must be mentioned that the infection to which the animals were submitted during pregnancy in this experiment was effected by instillation of a culture into the conjunctival sac*—a more effective way of infection than ingestion of the culture as employed in the first experiment.

The result of this experiment was that 16 of the 17 treated calves later calved normally, while one delivered a weak calf prematurely; in this case the placenta plus colostrum was infected with abortus bacilli. In addition, abortus bacilli were demonstrated in one of the animals that calved normally. Of the 16 controls seven aborted, and three delivered weak calves prematurely. By inoculation of guinea-pigs the presence of abortus bacilli was demonstrated in the uterine contents plus colostrum in eight of these ten animals; in the remaining two cases no search was made for abortus bacilli, as these abortions were not immediately observed. Among the six controls that apparently calved normally, abortus bacilli were demonstrated in the uterine contents plus colostrum in one case, and in the colostrum alone in another. Only in the remaining four controls that calved normally could no abortus bacilli be demonstrated anywhere.

In this experiment the same observation was made as before: that the immunity was retained in the following period of pregnancy.

In both of these experiments the vaccination was carried out with cultures of different virulence, and the findings appear to establish that a strain of relatively low virulence was preferable for such vaccination of calves.†

For various purposes—among others, in order to study certain details concerning the serum reactions, besides the effectivity of different ways of infection—Buck, Cotton and Smith have later supplemented their experiments on vaccination of calves. In October, 1938, a third report‡ was published, concerning 23 animals of which all the vaccinated were resistant to the later infection. Finally, Cotton read a paper§ at Hartford (Connecticut) in 1937, in which he gave a survey of all the American calf-vaccination experiments,

* This method for introducing infection, which has been adopted widely in experimental research in abortion, is usually stated in the literature to have been introduced by American investigators (Schroeder and Cotton (1924)). Long before that time, however, it was employed and described in investigations carried out in the Danish State Veterinary Serum Laboratory (*Skandinavisk Veterinär-Tidsskrift.* (1918). **8,** 250, and *Deutsche Tierärztliche Wochenschrift.* (1919). **27,** 124.

† Dr. Buck has been kind enough to supply the Serum Laboratory with such a strain (No. 19); it is at the disposal of other investigators who may want to use it for experiments.

‡ United States Department of Agriculture, Technical Bulletin. (1938). No. 658.

§ An abstract of this paper was kindly given the author by Dr. Eichhorn, Chief of the "Animal Disease Station," U.S.A.

including also a fourth, presumably unpublished, experimental series (20 animals) in which the infection dose was so large that *all* ten controls (*i.e.*, non-vaccinated) aborted, whereas only two of the ten vaccinated animals aborted, while the others were not infected at all.

The total American experimental results may be tabulated as follows:—

43 *Vaccinated Calves.*	*Per cent.*	33 *Controls.*	*Per cent.*
Abortion or premature delivery of weak calf in	7	Abortion or premature delivery of weak calf in	63·9
Normal delivery of viable strong calf in	93	Normal delivery of viable strong calf in	36·1
Complete resistance to infection (no abortus bacilli in milk or in uterus) in	80	Complete resistance to infection (no abortus bacilli in milk or in uterus) in	27·7

On the basis of these experiments Buck, Cotton and Smith recommend vaccination of the calves in strongly infected stocks, in which temporarily it is impracticable to cope with the disease by means of serological tests, sale of animals, isolation, etc.

In the lecture mentioned above Cotton stated that in U.S.A. about 3,000 calves, belonging to 250 infected stocks, had now been vaccinated in veterinary practice. The results are awaited with considerable interest, not least by American farmers, among whom the calf-vaccination experiments have attracted very great attention. Some authors (*e.g.*, R. R. Birch*) think that these experiments have attracted too much attention, as it is to be feared that the farmers will prefer vaccination to the ideal: combating of the disease to its complete eradication.

Writer's Experiments.

Even when I read the first report by Buck I wanted as soon as possible to try vaccination of calves in experiments carried out in veterinary practice under Danish conditions.

After looking around for some time I gradually succeeded in finding six fairly large farms that seemed to be suitable for such experiments. These experiments were commenced in autumn 1933, and they have been going on until now, when they are temporarily concluded. The farms were: Sanderumgaard, Ørnfeldt, Selleberg, Mullerup—all on the island of Funen—and the two estates, Hardenberg and Rosenlund, on Lolland. On all these farms infectious abortion had prevailed among the heifers in a severe or moderate degree throughout the year preceding the experiments; thus the case frequency on Rosenlund was 50 per cent, on Hardenberg about 25 per cent.

Originally I had wished to vaccinate only half of the calves on each farm and let the other half serve as control material. But this scheme could not be carried through. The owner insisted that more

* *The Cornell Veterinarian.* (1937). **27**, 161.

of the calves had to be treated, and the result was that about two-thirds of the calves were vaccinated, while only one third were allowed to serve as controls. Whether an animal was to be vaccinated or merely serve as control was largely a "catch question," as the calves usually had to be picked out from a pen. A record was made of each animal by marking it with a solid and distinct metal earmark (Hauptner's autocrotal mark, which is known to be very seldom lost).

The age of the animals has been from three to eight months when they entered the experimental period; most of them were from four to six months old.

Throughout the experimental period the vaccinated animals and the controls have been living together, under the same external conditions, and the covering has commenced at the same time for all of them.

As to the performance of the vaccination, I have followed the American principles. The original American, slightly virulent, strain No. 19, has been employed throughout the experiment. The "vaccine" (cultures) is prepared by suspension of well-developed agar-surface cultures (Roux flasks) in physiological salt solution. It was my intention, as was done in the American experiments, to employ the vaccine in a concentration of 10 × Tube 1 in McFarland's nephelometer, in doses from 3 to 10 c.c., depending upon the size of the calf (suggested by Dr. Buck). To start with, however, this nephelometer was not available to me, and I employed the earlier, presumably official American agglutination antigen as the basis for measuring of the concentration.* Later, after we had obtained a McFarland nephelometer, it was ascertained that our original dose, adjusted in the way just mentioned, was essentially greater than the doses employed in the American experiments. Within reasonable limits, however, the question of dosage appears to be of no importance to the immunisation, according to experiments carried out by Buck, Cotton and Smith.†

Each of the treated calves has received only one injection, subcutaneously. On the whole the calves have tolerated the injection well, although the fever has often been strikingly high for a couple of days after the injection. In some cases the temperature has been as high as 41·6° C. (107° F.), and this naturally is associated with debility and loss of appetite. No abscess-formation has been observed.

In cases where abortion has taken place the placenta has as far as possible been sent to the Serum Laboratory for further examination.

The results of the total experiment are given schematically in Table 1.

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* Tube 1 of the McFarland nephelometer corresponds to the agglutination antigen which now seems to be "official" in U.S.A.

† Dr. Eichhorn has kindly placed the manuscript for the paper at my disposal; I do not know where it has been published.

TABLE I.

SURVEY OF RESULTS IN EXPERIMENT ON VACCINATION OF CALVES AGAINST INFECTIOUS ABORTION.

Total Experimental Material: 486 calves.

Farms.	328 *Vaccinated.*				158 *Controls.*			
	Normal Calving.	*Abortion.*	*Uncertain Calving or "Abortion."*	*Sold, Died or Slaughtered, not Pregnant.*	*Normal Calving.*	*Abortion.*	*Uncertain Calving or "Abortion."*	*Sold, Died or Slaughtered, not Pregnant.*
Sanderumgaard ...	60	2	2	11	21	11	2	3
Ørnfeldt	39	1	–	27	16	9	1	8
Selleberg	30	–	4	1	12	4	1	2
Mullerup	15	(1 spirillum abortion)	–	4	1	1	–	3
Hardenberg	67	6	3	7	26	6	4	4
Rosenlund	33	–	4	11	13	3	4	3
Altogether ...	244	9 = **3.3%**	13 = 4·8%	61*	89	34 = **25·1%**	12 = 8·8	23†

* 31 on account of positive tuberculin reaction. † 8 on account of positive tuberculin reaction.

Table I shows, for one thing, that the percentage of abortion among the vaccinated animals has been 3·3, among the controls 25·1. In calculating these percentages, of course, the sold, killed, slaughtered, and non-pregnant animals have been left out.

Thus there is a very essential difference between the treated animals and the controls, which presumably justifies me in characterising the results of the vaccination as really good—and they are quite in keeping with the very promising American laboratory experiments, on the basis of which this experiment was undertaken. Naturally, it is not to be overlooked that the very low percentage of abortion among the treated animals has to be considered in relation to the fact that the abortion percentage has been comparatively low for the controls too, essentially lower than in the year prior to the commencement of the experiments. This relatively low abortion percentage for the controls may possibly be due in part to a decrease in the dissemination of the infectious agent in the houses because so few of the vaccinated animals aborted.

Of course, "experiments in the field" like those here presented imply the shortcoming that the expert observation of the animals naturally cannot be so efficient in the field as in a laboratory or experimental station, and this holds true in particular of the grazing periods. There are cases where the question has to be left open, whether or not an "abortion" (infection) has taken place. This applies to "normal" delivery of the calf with retention of the afterbirth, to deliveries that are a few weeks premature, and to still-born but otherwise normally developed calves, besides a few other special cases. With a view to this point, however, we have the control material for comparison, and, as shown in Table 1, the percentage of uncertain cases is twice as high for the controls as for the treated animals. So, really, it appears as if infection with abortus bacilli may have played a rather great rôle in these cases.

It has not been practicable to have the afterbirth of the "normal calvers" examined; and examination of the colostrum in the normal cases had also to be given up. Accordingly the true infection percentage cannot be calculated for the present material.

How the second period of pregnancy will turn out for these animals is something about which I shall not venture to say anything at present. For various reasons it will be considerably more difficult to obtain sufficient data to throw some light on this problem in practice.

On the basis of this experiment carried out by the Serum Laboratory in practice, together with the American laboratory experiments cited before, I think, there can be no doubt that future vaccination with living cultures against infectious abortion ought to be applied primarily to the calves. But such vaccination with living cultures must never become an "anodyne" or "sleeping-draught." For we cannot eradicate the disease radically by injection of cultures, and this applies also to vaccination of calves, which is

something that has been demonstrated plainly by the American experiments as well as the Danish. *Still, it is safe to recommend vaccination of calves as a valuable temporary means to reduce the number of abortions.*

On behalf of the Serum Laboratory and myself I wish here to render our best thanks to the veterinarians, Dr. Fr. Bay (Sakskøbing) and Dr. B. Voss (Rynkeby), for obtaining this material for us and for their ever ready assistance in carrying out the experiments.

In particular, moreover, we wish to acknowledge our indebtedness to our American colleagues, Dr. W. E. Cotton and Dr. Eichhorn for their general helpfulness in the performance of these experiments. It was not granted Dr. J. M. Buck to see the practical results of his comprehensive and fundamental studies on vaccination of calves—he died in May, 1938.

Summary.

A detailed account is given of a number of recent American laboratory experiments, showing that experimentally it is practicable, in dealing with infectious abortion in cattle, by vaccination of the calves at the age of four to six months, to get excellent results, covering several successive periods of pregnancy. This vaccination consisted in a *single* subcutaneous injection of living abortus bacilli of low virulence (strain Dr. Buck, No. 19).

During the years 1933-1938 the State Veterinary Serum Laboratory, Copenhagen, has carried out some field experiments after the American method, employing the American culture strain for preparation of the vaccine. These experiments comprised altogether 486 calves belonging to the stocks of six large farms. About two-thirds of these calves were vaccinated, while one-third served as controls. The experimental animals and the controls were picked out at random, and they have lived together—that is, under the same conditions—throughout the experimental period.

The results are given in Table I, showing that the percentage of abortion for all the treated animals has been 3·3, whereas for the controls it was 25·1.

It is pointed out that future vaccination against infectious abortion in cattle with employment of living cultures ought to be applied to the calves. But vaccination of the calves is not a goal to be aimed at, as the disease is not eradicated thoroughly in this way. Gradually, as the conditions in the individual stocks and districts allow it, the combating of this disease ought to go on to diagnosis through blood tests, sale (isolation) of infected animals and disinfection—just as in the combating of tuberculosis.

THE SKIN LESIONS OF BOVINE TUBERCULIN REACTORS, SECOND REPORT

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Introduction.

In presenting our second report we have first to admit failure of our attempts both to cultivate a causal agent of the lesions* and to transmit the condition by experimental animal inoculation. We have, however, accumulated additional data and material, which may be of assistance in further investigations on this important subject.

Since the publication of the preliminary report[1] from this laboratory, a number of fresh cases of " skin lesions " have been brought to our notice. Eighteen of these animals have been purchased (or presented), tested with tuberculin at the laboratory, and finally each one subjected to a *post-mortem* examination in which the search for lesions was as complete and thorough as we could make it. All the lymph glands we could find were removed from the carcase, sliced thinly, and examined for macroscopic lesions ; the glands were divided into a number of groups, and representative material from each group was emulsified in salt solution and inoculated into guinea-pigs and fowls ; the viscera and carcases were carefully inspected, and finally the vertebral column was split or sawn down and examined for lesions.

The results of our examinations and experimental inoculations are the subject of this report, and in addition there are descriptions of materials sent to us for diagnosis, and of some experimental inoculations we have made with known acid-fast organisms. Publications that have come to our notice since our previous report in 1937 are recorded in a bibliography.

Geographical Distribution.

We cannot pretend to give an accurate survey of the incidence of the condition in Great Britain, firstly because all cases are not reported to us, and secondly because in only a few reported cases are we able to make confirmatory diagnoses. The latter point is of some importance, because although one may be confident in clinically diagnosing typical cases such as shown in Figs. 1, 2 and 3, isolated lesions present more difficulty. Myxo-fibromata and fibromata have been met with in front of the shoulder, about walnut size and very often with skin attachment ; one case of suspected teat lesions has been examined and proved to be a tumour. Then there is Nodular Necrosis of the Muscle[2] which in an early or less generalised

* See addendum, p. 215.

stage is not easy to differentiate, for although these lesions are intramuscular in position it is very difficult to tell by palpation if a nodule is attached to subcutaneous fascia or lying in the panniculus muscle. It is a very simple matter, however, to enucleate a small skin nodule and send it for diagnosis.

The map in Fig. 24 records the source of every case we have confirmed here. We do not know whether the disease originated on the farm from whence it came, because we do not know the incubation and developmental periods. It will be seen that the condition has a wide distribution in Great Britain. The number of cases brought to our notice is increasing ; not, we think, because of an increased incidence, but because of the greater attention now being paid to tuberculosis eradication.

Andersson[3] has described the condition as an infectious disease and states that " outbreaks " may occur. His evidence on this point is meagre. Our early experiences might have supported his theory on the grounds that nearly all our cases came from one estate, but lately we have confirmed cases from many parts of the country, and if infectious the etiological agent must have a wide distribution throughout Great Britain.

The Lesions.

The commonest site of lesions is undoubtedly the legs, and in our experience more often the forelegs. Of the 30 animals with lesions that have been thoroughly inspected at this laboratory, 25 have had lesions on the fore limbs, seven lesions on the hind limbs, one a lesion on the flank, one a lesion on the back, and one lesions on the neck ; only three animals have shown lesions on the hind limbs without fore-limb involvement.

The position of the lesions, particularly on the fore limbs, is very uniform. They nearly always lie along a line, presumably following superficial lymphatic vessels, which descends from the prescapular lymph gland along the antero-lateral aspect of the upper leg as far as the elbow joint, then passes obliquely round the lateral aspect of the radius to behind the carpus (Figs. 1 and 2). Lesions in the regions of the metacarpus and the lower fore leg joints have only once been seen by us (Fig. 4). When two or three lesions only are present, they generally lie in the carpal region, but they may occur as isolated nodules higher up the leg. We have positive evidence in one case at least (Fig. 1) that the first lesions to appear were in the carpal region, and subsequent nodules developed successively in the ascending direction. This " line " distribution on the foreleg has also been recorded by Andersson in Scandinavia[3] and the centripetal direction of development is mentioned by Mohler[4]. Two favourite sites for hind limb lesions appear to be about the lateral aspect of the hock, and the lateral aspect of the metatarsus and the fetlock joint. (Fig. 3.)

In character the lesions are non-painful, cold, and as a general rule, firm, rounded swellings, but they may, especially on the hind

limbs, be thick-walled abscesses that may be fluctuate on firm, digital pressure. They may be quite free from the overlying skin, or adherent to it with actual intradermal involvement, in which case superficial lesions may always be found in the form of ulcers, hæmorrhagic crusts, loss of hair, or healed scars. Lesions of the softer abscess type may be discharging.

In size the lesions seen have varied from a small pea to a tangerine, the softer abscess types sometimes reaching even greater dimensions ; confluent masses of nodules may also occur and form prominent areas of nodular thickening of considerable size (Figs. 2 and 4). In one case such a confluent mass formed a complete covering round the posterior aspect of the first phalanx.

The macroscopic appearance of the sectioned lesions is variable and we now recognise three types ; one or all types may be present in the same animal, but type 3 frequently occurs alone. In the first type the nodules are made up of living tissue, of firm consistency and characteristic light brown colour, sometimes but not always showing small foci of necrosis. There always appears to be skin involvement with this type, careful sectioning revealing at least one spot in which the light brown tissue has replaced the normal white colour of the dermis and reached and penetrated the epithelium (Fig. 6). Type 2 is subcutaneous in position, and is formed of a tough capsule enclosing firm yellow granular-looking necrotic material, frequently quite dry and often gritty to the knife. The walls of these cavities may be thin and fibrous, or may show variable thickenings of the light brown tissue of type 1. Type 3 is the abscess type commonly seen on the hind legs, and is similar to type 2 except that the contents are fluid, creamy or creamy yellow pus of varying consistency. These abscesses always burst at some time in their development and discharge their contents to the surface. Afterwards more of the light brown tissue appears to develop, and the lesion becomes less prominent though more solid ; pus, however, may form again and another discharge occur.

The histological appearance of sections of nodules of the three types varies only in the amount and character of the necrosis. The essential granulomatous tissue is present in varying amount in all cases, giant cells being always present, usually in considerable number. The histological distinctions are not greater than those seen in tuberculous lesions of different stages of development, activity, or regression.

The intimate histology is described in a later paragraph, but in view of the considerable structural variations seen in tuberculosis it does not appear to us to be possible to differentiate these skin lesions on histological grounds from tuberculous granulomata. The significance of this similarity is not so impressive if it is remembered that saprophytic acid-fasts can be made to produce granulomatous lesions in guinea-pigs, as shown by Hagan and Levine[4], and in and under the skin of cattle, as is described later in this article.

Lymphatic Gland Lesions.

We have noted in several cases that the lymphatic gland draining the affected area of skin was larger than normal. On section these glands were firm and showed a homogeneous cut surface, sometimes flecked with a few irregular haemorrhages, but with one or possibly two exceptions nothing was seen that could be described as a macroscopic lesion. In all except our earlier cases histological examinations of the lymph glands adjacent to the skin lesions have been carried out. We found that where enlargement is seen macroscopically histological lesions are present. In general these take the form of a rather diffuse epithelioid cell invasion, and the appearance of such sections suggests that the structural changes tend to follow or to originate from the capsule trabeculæ penetrating the lymphoid tissue. This is what might be expected if the infective agent is taken to the gland by the afferent vessels.

Case 30 was the first in which we recognised macroscopic lesions in a lymphatic gland. This beast showed a number of lesions in a fore limb, following the usual " lymphatic line." The upper nodules dipped into the superficial muscle, and one lesion was situated on the very margin of the gland. The gland itself was enlarged and two hard centres could be felt in its substance. On section the hard centres were found to be rather gritty necrotic lesions, with a macroscopic appearance we could not distinguish from that of tuberculosis.

The lesions in the prescapular gland of Case 15 might well have been an extension from the skin lesions, which occurred right up the fore limb of the same side. Unfortunately they were considered at that time to be connected with the tuberculous lesions present in a mediastinal gland, and cultural and biological tests were omitted and sections were not made.

The structure of these lymph gland lesions is considered under histology.

Krantz, in 1938[5] reports histological lesions of the regional lymph nodes in 12 out of 32 examinations made. In seven cases he was able to demonstrate acid-fast bacilli in these lesions. We have found acid-fast organisms in the gland lesion of Case 30, and in Case 15 (if a skin extension and not true tuberculosis), but otherwise our examinations of gland smears, including the five in which histological lesions only were found, have been negative.

Cases of Special Interest.

(*a*) *Co-existent tuberculosis.*—We have found two cases of skin lesions in animals in which the mediastinal glands showed lesions of tuberculosis (Cases 15 and 21, appendix). Biological tests proved the organism to be a bovine type. Two further skin lesion cases (Nos. 26 and 28) showed lesions in the mesenteric glands, macroscopically characteristic of avian tuberculosis. Acid-fast organisms were present which have in one instance proved to be of the avian type ; biological tests with the other material produced no lesions

in fowls or guinea-pigs, but the inoculated animals reacted to avian tuberculin.

Except that Wright[6] found that 50 per cent. of the cases he examined had tuberculous lesions, skin lesions in tuberculous animals do not appear to have been frequently reported. This does not necessarily indicate that skin lesions are most common in non-tuberculous animals, because it is in the clean herds that cases have been brought to light owing to unexpected tuberculin test results. The finding of avian and bovine infections in addition to skin lesions does suggest that there is very little or no cross-immunity between *M. tuberculosis* and the skin lesion factor, and also that unless the tuberculous lesions are extensions from the skin, or *vice versa*, that the skin lesions are not caused by either avian or bovine tubercle bacilli.

(*b*) *Muscle lesions.*—Lesions situated in the muscle were first seen in Case 31, a specimen sent to us for diagnosis. The nodules occurred in front of the shoulder, and were embedded in the muscle instead of lying in the usual subcutaneous or intradermal position. They were separated from the muscle tissue by a fibrous capsule, which appeared to be an extension of the interfasicular connective tissue of the muscle. The lesions were mainly of type 3 with thick pus, and acid-fast organisms were scantily present in smears. Histologically the necrotic centre was enclosed by a zone of lymphocytes, and a few centres of granulomatous tissue with giant cells were scattered in this zone. Plasma cells were very numerous, and there was some infiltration of the muscle tissue proper by lymphocytes, but there was no evidence of granuloma formation outside the boundaries of the fibrous capsule. The macroscopic and microscopic appearances were quite distinct from those of Nodular Necrosis of the Muscle.[2] Animal inoculations carried out with this material gave entirely negative results. No other lesions of any kind were found in this animal at a field *post-mortem* examination.

(*c*) *Spontaneous absorption of skin lesions.*—This phenomenon has been noted before, and Andersson[3] finds it quite common. We have observed lesions to diminish in size in so far as the original field descriptions of size have not agreed with our findings, and naturally where pointing and discharge of the contents has occurred with type 3 lesions the size is considerably reduced. In only one case, however, have we seen lesions completely disappear. Case 17 was an animal originally purchased for an abortion experiment, and when received was noted to have skin lesions on one fore leg. These were quite well defined, the largest being the size of a hazel-nut. The animal was used in the abortion experiment for nearly 200 days, during which period it was noted that the lesions were steadily diminishing in size, until at *post-mortem* examination nothing could be felt or seen, except that very careful sectioning of the skin over the area where lesions were known to have occurred revealed some faint scarring of the dermis with light brown tissue. Sections examined microscopically showed the typical appearance of skin lesions type 1, except that giant cells were not seen.

Discussion of the Lesions.

We have not always recognised three types of lesion, and we have no evidence to prove that there is an etiological factor common to all three. Acid-fast organisms of a similar appearance may, however, be found in smears made from any of the three types, and the fact that there exists a histological similarity and that they all appear capable of provoking a tuberculin reaction at some time leads us to assume that they are different forms or stages in development of one condition. Type 3 has only recently been observed by us, and when we were dealing with types 1 and 2 we decided that type 1, with the superficial lesions, might well represent the original infection and the mode of entry of the organism, and that type 2 was the sequel, with encapsulation and necrosis of the infected tissue. Krantz[5] expresses the opinion that infection does occur through the skin. The examination of Case 16, with the prominent scar (Fig. 5) resembling a hedge tear, tended to confirm this idea, but the finding of type 3 cases has upset our premises. Type 3 cases point and discharge so frequently that we now feel that the surface lesion of type 1 must be regarded as evidence of previous pointing, not as the site of entry of infection, if the three types are stages of development of one condition ; that after pointing the infection spreads into the layers of the skin and causes the formation of granulomatous tissue, and that the lesions of type 2 are those that have never reached the surface and in which the pus has become inspissated. Most if not all of the lesions we have seen are at least six months old. The position of the lesions and the centripetal direction suggest a lymphatic dissemination, but the original site of entry of infection has in our opinion not yet been revealed. While it would certainly appear probable that the responsible factor gains access through the skin, since lesions tend to develop from the extremities towards the trunk, we do not think the evidence we have examined indicates infection through the skin over the site of the lesions ; injuries to the foot must be considered as possible portals of entry.

The Tuberculin Test.

Wherever possible we have obtained the complete records of all tuberculin tests carried out on the animals affected with skin lesions. Fig. 25 is a table in which we have attempted to show clearly and simply the course and size of reactions in successive tests. Under " Tuberculin Reactions " the time interval between the tests is shown, and under " Test Measurements " the increase in skin thickness at the 72nd hour is shown to scale. Of course we realise that measurement is not the only criterion by which reactions are judged, but they do undoubtedly form a very good guide.

The value of measurement is much disputed at the present time. We are of the opinion that it is a guide to the severity of the reaction. However, we are not sure whether we are correct in Table 25 in showing the increase in skin measurement as a unit of the original skin

thickness. Is the response of a diffuse thickening of 4 millimetres the same with a four millimetre thick skin as with a 12 millimetre thick skin ? We have examined the results and measurements of a number of tuberculin tests in this country, and ignoring for the moment the physiological details such as oedema, it would appear that in a high percentage of cases an increase of thickness equal to or more than that of the original skin is found in animals recorded as positive ; doubtful reactors usually show a thickening of more than 1/2, and less than the original measurement, and below the 1/2 the results are negative. We repeat that we are not suggesting that measurement is used as the sole guide, but in the example we have quoted an increase of 4 mm. on a 4 mm. skin with slight œdema would probably be read as positive, whereas with a 12 mm. skin the equivalent amount of œdema would be more diffused and more difficult to detect, the skin increase being only 1/3 of the original thickness, and the result would probably be read as negative. Yet the amount of response was the same in both cases.

We have used in this article a fractional method of expressing skin readings, which is simple and shows both the increase (the numerator) and the original skin thickness (the denominator).

It was pointed out in our preliminary report that American experience in general indicates (*a*) that the reaction of skin cases to tuberculin is usually less marked than that of tuberculous animals, and (*b*) that on retest a number fail to react at all.

(*a*) We are now of the opinion that in this country quite large positive reactions may be obtained in skin lesion cases which on *post-mortem* and biological examinations show no other lesions at all, particularly, in some cases with avian tuberculin and in others with both avian and bovine tuberculins. In the four cases in which we have found co-existent tuberculous lesions the reactions were not markedly more prominent than those in some of our " no other lesion " skin cases. We are unable to draw any parallel, from our results, between the size of the reaction and the number or volume of lesions found. Andersson[3] has made a similar observation. Case 27, for example, with large lesions of the pus type and very numerous clumps of organisms, never reacted to either bovine or avian tuberculins, while in Case 17 the reactions increased in size as the lesions were absorbed, and a definite positive response was given to bovine tuberculin when no lesions could be seen or felt.

(*b*) It will be seen from our table that only a very small percentage of our cases have failed to react to one or more retests with tuberculin.

It must be remembered that in America a single tuberculin test is used, not a double injection as in this country. This fact might account for our reactions with skin cases being larger. It is not proposed to enter into a discussion of single versus double injections ; in our personal laboratory experience we consider that reactions can be read at the 48th hour. There is no doubt, however, that the second injection does increase the reading when taken at the 72nd hour. If, on the other hand, the failure of skin cases to react to a

retest is explained on the grounds that the amount of antibody stimulation by the lesions is so small that it is neutralised by the single injection of tuberculin, it would be expected that a double injection would have an even more marked effect in this respect. It has been suggested that a reactor can be made to give a negative test by injecting a volume of tuberculin shortly before the test is carried out, and Case 25 is an example (of a " skin " case) of the effect of an overdose of tuberculin on a subsequent test. This animal was tested with three different brands of bovine tuberculin at the same time, and gave a well-marked reaction to all three ; 22 days later when retested it gave a very definite negative to both avian and bovine tuberculins ; numerous skin lesions, some very large, were present.

Another rather interesting feature of the repeated tests is the frequent occurrence of animals that react to bovine tuberculin in the field, and when retested do not react to bovine but give a marked reaction to avian tuberculin. It has been suggested to us that the acid-fast group must have a complex antigenic structure, including both group and specific components. This may well explain a number of puzzling results in tuberculin testing ; there must be some reason why some animals react to bovine tuberculin and not to avian, or *vice versa*, whilst others give a very well marked reaction to both avian and bovine tests (Cases 6, 19, 26, 30).

Mohler[4] remarks that sensitisation to the tuberculin test continues as long as new skin lesions are forming. If these lesions are responsible for the sensitisation our results indicate that the tuberculin reaction may persist during and after the disappearance of the lesions, as is seen in Case 17.

The study of the tuberculin sensitivity of skin cases is very far from complete. We have in view an experiment in which a number of cases will be examined and tested over a long period. One point on which we have no information at all is how soon after (or before) the appearance of lesions animals react to tuberculin ? Attempts to obtain information about the first appearance of lesions have with very few exceptions proved fruitless.

Finally it must be emphasised that at present there is no proof that the skin lesions or the acid-fast organisms they contain are responsible for the tuberculin sensitisation. Our attention is usually drawn to skin lesions not because they are skin lesions but because an unexpected result has been obtained from a tuberculin test. The failure to elicit any reaction at any time from an animal in which a large number of acid-fast organisms were present (such as our Case 27) needs explanation. A survey of a large number of skin-lesion cases, detected independently of the tuberculin test, is really necessary before we are justified in the positive linking of skin lesions with tuberculin reactions.

The Acid-fast Organism.

Descriptions of skin lesion cases are uniform in that all investigators describe the presence of acid-alcohol fast organisms in the

lesions. With one exception we have in every case been able to demonstrate such an organism in smears prepared from the lesions and stained by the Ziehl-Neelsen method.

The form and staining properties of these organisms vary very considerably. As a general rule they seem to be broader than the tubercle bacillus and stain a darker red, but we have had cases in which differentiation from *M. tuberculosis* on morphological grounds was, in our opinion, impossible.

Curved forms, beaded forms, and a very small bipolar-staining form are commonly seen. The impression we have from the appearance of a large proportion of our smears is that the organisms are degenerating and breaking up.

In number we have usually found them scanty, but in some instances they were numerous, and in Case 27 they were very numerous at one stage of lesion development. This animal when first seen had a large lesion of the soft abscess type below the fetlock on a hind leg. The abscess was lanced, and pus taken for examination, culture, and animal inoculations. Examinations of smears of the pus showed very numerous organisms, and there were many clumps, as shown in Fig. 7a, suggesting that actual colonies of acid-fasts were present in the pus. We were very disappointed that our cultural and animal inoculation attempts with this material proved entirely negative. When this animal came for *post-mortem* examination, the abscesses had discharged, there was little pus but considerable granulation tissue, and acid-fast organisms were very scarce in smears.

Attempts at the culture *in vitro* of this organism have been repeatedly made, under aerobic, anaerobic, and microaerophilic conditions, both at room temperature and at 37° C. The following media have been used : plain egg, glycerine egg, plain agar, glycerine agar, Sabouraud's agar, serum agar, agar combined with extract of bovine epithelium, Twort's medium and Löwenstein's medium.

The results have been uniformly negative. It is interesting to note that when the inoculum has been taken and prepared in a sterile manner we have never obtained a growth of any organism at all, even from the pus of type 3 material. It must be noted that at present no one has proved that these acid-fast organisms are responsible for the development of the skin lesions.

Biological Examination of Skin Lesion Materials.

Preparation of Inocula.—The contents of the skin nodules and scrapings and tissue from the walls of the lesions were in each case taken with aseptic precautions, triturated without sand in a sterile mortar and suspended in a small quantity of sterile normal saline solution. The suspensions were filtered through glass wool to remove the coarser particles before inoculation. In a number of cases liquid paraffin was used as the excipient in place of normal saline, and in these the suspension was shaken for several hours in a mechanical

shaker to obtain an even distribution of the particles, and the inoculum was warmed in the incubator at 37° C. to facilitate inoculation.

The reason for using the oily excipient was because of the work of Hagan and Levine[7] and a number of unpublished experiments of our own on the small laboratory animals, which show that members of the acid-fast group, whether pathogenic or saprophytic, alive or dead, will produce lesions when inoculated in an oily suspension. Our results have not agreed entirely with those of Hagan and Levine in that we have not obtained lung lesions in guinea-pigs following subcutaneous inoculations in nearly such a high percentage of cases as they have, but we have always obtained marked lesions in the local lymphatic glands.

Animals employed.—For routine inoculations with normal saline suspensions we have used six guinea-pigs (three receiving 2 c.c. subcutaneously and three receiving 2 c.c. intraperitoneally), two rabbits (2 c.c. subcutaneously and 0·5 c.c. intravenously respectively), and two or four tuberculin-tested fowls (intramuscular and intramuscular plus intravenous injections). The fowls have always been tested with avian tuberculin prior to killing for *post-mortem* examination, and in the more recent experiments the guinea-pigs and rabbits have been tested with both avian and bovine tuberculins immediately before death.

Guinea-pigs and rabbits inoculated by the intraperitoneal or intravenous routes have been examined eight weeks after inoculation, and those inoculated subcutaneously after a lapse of 12 weeks ; the fowls receiving intramuscular plus intravenous injections were examined after 12 weeks, and those receiving only intramuscular injections after 16 weeks.

In addition to these routine inoculations material from a number of cases has been inoculated subcutaneously and intradermally into tuberculin-tested calves, in both saline and oily excipients. Rabbits, guinea-pigs and fowls have received liquid paraffin suspensions by the subcutaneous, intravenous and intraperitoneal routes. After the report by Wells[8] a small number of field voles were used for skin material inoculations.

Each experimental animal has received a careful *post-mortem* examination and cultural and histological examinations have been carried out, with any abnormalities noted in their tissues. In no case have we been able to produce lesions of any significance, nor have we been able to record any degree of sensitisation to tuberculin.

From the fact that our results with liquid paraffin suspensions have been entirely negative, it might be assumed that the acid-fast organism does not belong to the Mycobacterium group. It must be remembered however that neither Hagan and Levine[7] nor ourselves have worked out the minimal dose of organisms required to produce lesions. In rabbits we have constantly obtained lung lesions and usually kidney lesions, from which the organism has been recovered in culture, by the intravenous inoculation of 0·025 mg. (moist weight) of *M. phlei*, *M. smegmatis* or *M. butteri* suspended in liquid paraffin.

The number of organisms injected in the skin-lesion suspensions is however very small, and if they are dead might well be insufficient to produce lesions. Until more thorough titrations have been made with the oily suspensions we do not feel justified in using our failures as grounds for dissociating the acid-fast organism of skin lesions from the Mycobacterium group.

Skin Lesions produced experimentally by known Acid-fast organisms.

We have employed bovine tubercle bacilli, avian tubercle bacilli, and Timothy grass bacilli for experimental intradermal and subcutaneous inoculations in cattle. Each organism has been inoculated in a watery excipient (normal saline) and in an oily excipient (liquid paraffin).

Tubercle bacilli of bovine or avian type promote the formation of a granulomatous nodule at the site of inoculation. The reaction to the bovine type of organism is, as would be expected, greater than that to the avian type. In both cases, however, whatever the excipient used, lesions occurred rapidly in the nearest lymphatic gland, and persisted there throughout the period of observation. The violence of reaction and the degree of spread must presumably be dependent on the virulence of the experimental organisms used ; those used by us were ordinary stock strains. There was no evidence of any formation of secondary skin lesions, even in animals kept under observation for six months. The infection appeared to pass to the nearest lymphatic gland without leaving evidence along its route. The glands showed macroscopic lesions as early as one month after inoculation. The histology of these artificial lesions was not identical with that of natural skin lesions. The normal variability of tuberculous lesions is such that we are chary of ascribing too great a significance to the differences. The few artificial tuberculous lesions we have examined, however, did show a marked granulocytic reaction, compared to the skin lesions proper in which granulocytes are not conspicuous. The same is true of the only natural case of true tuberculosis of the skin we have examined* ; here the granular leucocytes were very prominent indeed, but as the lesions were open they may have been a reaction to secondary infections.

Inoculation of *M. phlei* in normal saline gave rise to no lesions. In the oily excipient, however, this organism gave rise to marked reactions at the sites of inoculation, and spread to the adjacent lymphatic glands. The histological picture was similar to that seen with the tubercle organisms, except that necrosis was rarely seen. The organism was readily recovered in culture from the lymphatic glands. As with the tubercle experiments, no trace of secondary nodule formation was found.

Although not pertinent to this particular problem, our results

* This is a misnomer ; the case was one of scrofulous cervical and submaxillary glands which had ulcerated to the surface and were discharging. Not a natural case of cutaneous tuberculosis.

with oily excipients are rather interesting in view of the report by Buxton and Glover[9], who inoculated B.C.G. in oily excipients (liquid paraffin or olive oil) by the subcutaneous route. Although doses as large as 50 mgm. were used, no lesions other than a local reaction to the liquid paraffin were found at *post-mortem* approximately nine months later. Yet we, inoculating a much smaller dose of an organism which produces no lesions at all in normal saline suspension, found the nearest lymphatic gland approximately three times the normal size, showing naked eye lesions on its cut surface, and containing numerous acid-fast organisms five months after inoculation.

Summarising our experiments with known acid-fast organisms, we have found no difficulty in reproducing solitary nodules simulating those of " skin lesions," but have not succeeded in producing a chain of lesions from one inoculation, neither have we been able to infect the skin without infecting the neighbouring lymphatic gland.

Histology.

Natural skin lesions.—On macroscopical grounds we have divided the nodules into three types. Type 2 and type 3 lesions have a very similar histological appearance, differing only in the nature of their necrotic contents, but type 1 lesions require a separate description.

The general picture of type 1 lesions is that of granulomatous tissue made up chiefly of lymphocytes, mononuclear leucocytes, plasma cells and epithelioid cells, with a variable amount of new fibrous tissue (Figs. 8, 9 and 12). Granular leucocytes are scanty, and although there is usually some necrosis it is present only as small centres. Giant cells are present in very large numbers. There is in general little of the focal character that is usual with tuberculous lesions, but the granulomatous tissue may form irregular nests. There is a plentiful formation of new vessels, which may show obliterative changes, endarteritis and periarteritis (Fig. 13). Except for the presence of numerous giant cells and occasional necrosis, the lesions in many respects resemble Muir's description[10] of a primary syphilitic chancre. The cellular infiltrations may follow the vessels into the deeper layers of dermis or subcutaneous tissue and small islands may be found a considerable distance from the major lesions (Fig. 11).

The epithelium over a lesion is much thickened and there is increased papillation of the underlying dermis. The hair follicles and the sebaceous and sweat glands are destroyed, and lesions which penetrate the epidermis appear to do so through infected hair follicles (Figs. 8 and 9). The epithelium dips deeply into the dermis, and encloses a core of cornified-looking material, which penetrates into the dermis at the base of the depression. In size these lesions vary considerably, some being very large, but we think that they are most probably distended hair follicles, an advanced stage of the early infection shown in Fig. 9. The " scar " lesion of Case 16, illustrated in Fig. 5, proved to be a large example with this structure. The epidermis in general appears to be very resistant, masses of the granulomatous tissue frequently lying under an apparently normal

epidermal layer, except that the stratum malpighi is in a very active state. Sometimes the epidermis is infiltrated with blood, the corpuscles being packed between the cell layers. The epithelium of root sheaths offers great resistance to the destructive processes of the granulomatous growth.

Type 2 and type 3 lesions present a rather different picture (Figs. 14 and 15). These lesions are mainly subcutaneous in position, but may extend into the deeper layers of the dermis. The essential feature of both is multiple centres of necrosis in different stages of liquefaction, caseation, or calcification. Granular leucocytes are more numerous than in type 1 lesions. The tissue surrounding these foci is again granulomatous, but with a more zonal arrangement than is present in type 1 lesions. Giant cells are numerous and are frequently seen undergoing destruction in the margins of the necrotic centres. The contents of the necrotic centres of type 3 lesions are usually so liquid that they drop out of sections in their preparation and only the granulomatous wall remains.

Experimental skin infections.—Lesions set up by the subcutaneous injection of the avian type of tubercle bacillus are histologically not unlike those of skin lesions types 2 and 3, but the necrotic centres contain masses of granular leucocytes, particularly eosinophiles (Fig. 19). The injection of the bovine organism resulted in extensive areas of necrosis margined by a regular zonal arrangement of granulomatous cells (Fig. 18). The necrotic masses contained many degenerating leucocytes, and extensive areas of haemorrhage. Both these organisms, applied by scarification, caused lesions more like type 1 skin material but with a marked eosinophilia. The " hair follicle " lesions were also present (Fig. 10). *M. phlei* in an oily excipient gave rise to similar pictures with marked zonal arrangement of the cells and numbers of granulocytes infiltrating the lesions (Fig. 20). Caseation occasionally occurred, but frequently there was no necrosis even after several months. Liquid paraffin alone sets up a diffuse inflammation of the injected areas ; the spaces left by the oil are clearly marked (Fig. 21). The reaction is mainly histiocytic, we have not seen necrosis following such injections, but giant cells may occasionally occur. The type of reaction and the rate of absorption of the liquid paraffin appear to vary very considerably with the individual.

Lymphatic gland lesions.—The histological changes seen usually consist of scattered nests of epithelioid cells in otherwise apparently normal lymphoid tissue. The lesions frequently appear to be connected with the trabeculae penetrating the gland substance. With the exception of the case with macroscopic lesions, giant cells, necrosis or caseation have not been seen. Indeed in many respects the lesions look more like proliferative changes of the reticular tissue of the gland than granulomatous formations. In Case 30 the lesions were composed of necrotic masses with very little reaction immediately around their margin. The gland was cirrhotic and irregularly distributed islands of epithelioid cells occurred in its substance (Fig. 23).

One giant cell only was found and that occurred in a necrotic area and was itself degenerating. A section of a portion of the gland not macroscopically abnormal showed a disseminated epithelioid invasion not unlike that seen in a mesenteric lymph gland in Johne's disease. The whole gland substance was invaded by an abnormal number of leucocytes of the granular type. (The skin lesions themselves in this case were of type 2 and type 3, and the fibrous walls showed very little of the granulomatous tissue of type 1.)

Bibliography.

Few publications have come to our notice since our first report[1]. Krantz[5] has described 40 cases on the island of Gotland, and he found, in addition to the skin lesions, microscopic granulomata in the regional lymph nodes of a number of his cases. His cultural and biological tests were all negative. Anderson[3] describes the condition on a number of farms, also in Gotland, Sweden. His descriptions of the lesions tally very closely with ours ; he describes the " lymphatic line," and the abscess type of lesion which may discharge. In his experience spontaneous absorption of the lesions seems to be quite a common sequel. He believes the condition to be infectious, and is quite certain that bovine tubercle bacilli are not responsible. There are many points of similarity between these skin lesions and Benign Lymphogranulomata (Boeck's sarcoid) as described by Lindau[11]. Weak tuberculin reactions are sometimes recorded in lymphogranulomatous cases, acid-fast organisms have occasionally been described, and the lesions have a typical tuberculous structure except that there is no caseating necrosis ; biological and cultural tests have proved entirely negative. However, such lesions in man may have a general distribution and affect the nasal mucous membrane, conjunctiva, lymph nodes, lungs, and bone, as well as the skin.

Nieland[12] and Potel[13] have described some cases of tuberculosis in the horse with rhinitis and skin lesions. In one animal there was tuberculosis in a mesenteric gland, but in the other two no other lesions were found. Cultural and biological tests were not carried out, but the condition was considered to be tuberculosis on histological grounds ; it seems quite possible, however, that they may have been cases of " skin lesions " in the horse.

Further investigations of " skin-lesion " cases are reported by Mohler in the Reports of the Bureau of Animal Industry[4, 14]. Apparently no success has been obtained from attempts at culture or experimental inoculation. In the 1935 Report[14] an experiment in which bovines with scarified feet were put on ground that had been spread four months previously with living bovine tubercle organisms resulted in negative tuberculin reactions and no lesion formation after more than one year's exposure. (The soil was not infective when exposure commenced.)

The condition known as Bumble Foot in fowls is of comparative interest. This disease has been regarded as a form of avian tuberculosis, and acid-fast organisms are demonstrable in the lesions of a

FIG. 1. Single lesions of large size on a fore limb, showing the "line" they so frequently follow. Case 26.

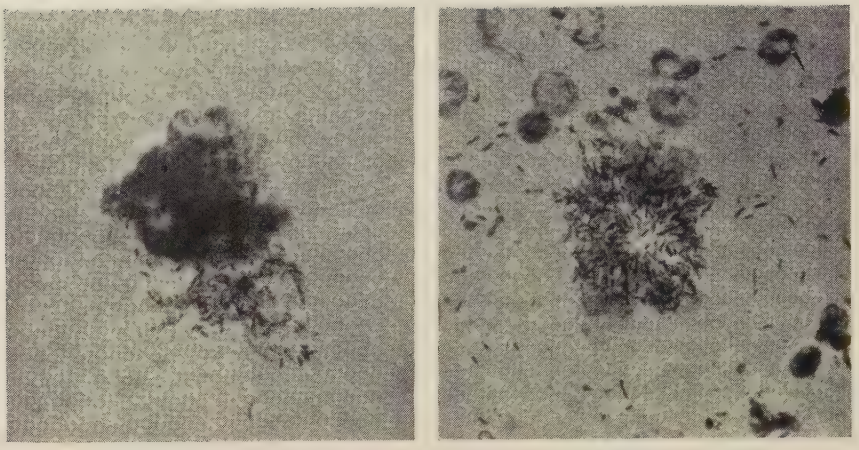
FIG. 2. Another severe involvement of a fore limb, showing more numerous smaller lesions and the formation of confluent masses. Case 24.

FIG. 3. A common site of lesions on a hind limb.

FIG. 4. Massive lesions of the subcutaneous tissues at the back and sides of the 1st phalanx. Case 32.

FIG. 5. The " scar " lesion of Case 16.

FIG. 6. Type 1 skin lesion. The amount of ulceration is unusually severe; more frequently there is only a bald patch on the hide.



(a) (b)

FIG. 7. (a) A clump of skin-lesion acid-fast organisms (pus from Case 27). (b) A clump of avian tubercle bacilli (spleen smear). Z.N. × 1200.

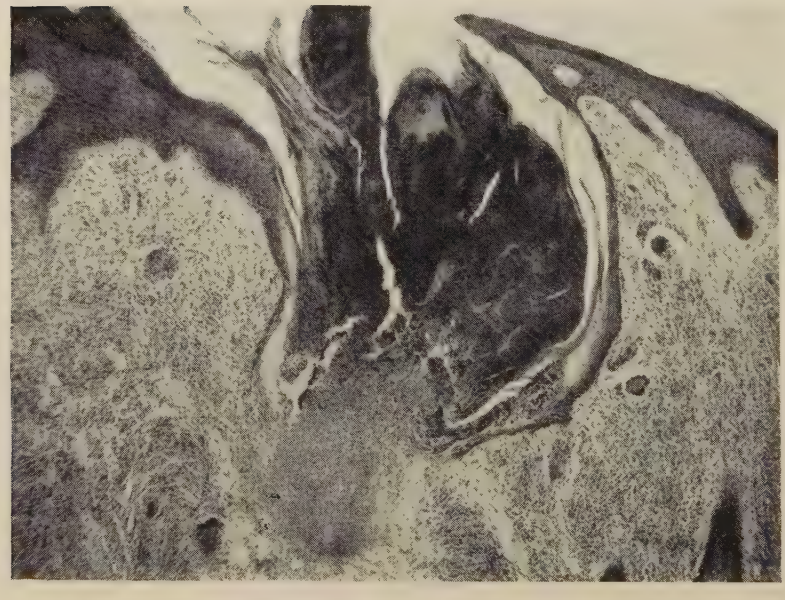


FIG. 8. Superficial lesions from a type 1 case. Necrosis at base of ? infected hair follicle. × 70.

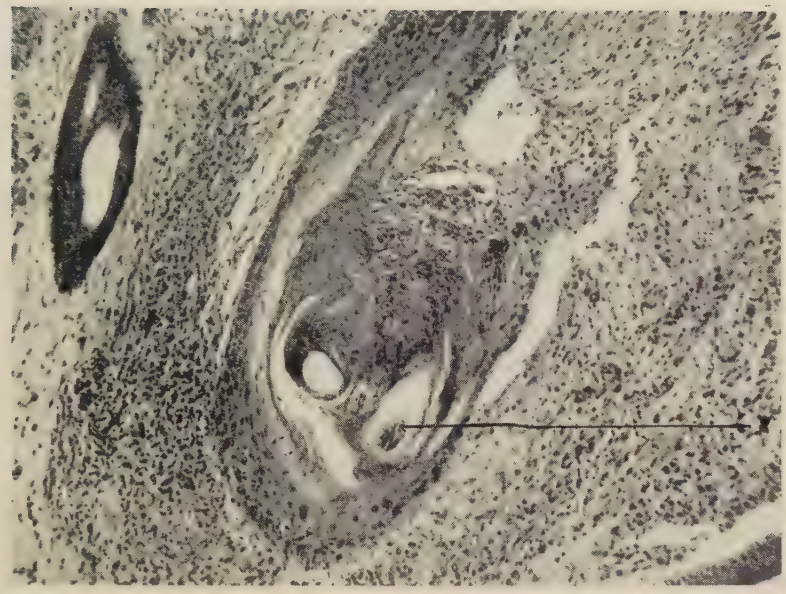


FIG. 9. An early infection of a hair root. The section is oblique and the remains of the hair proper is seen at "x". × 120.

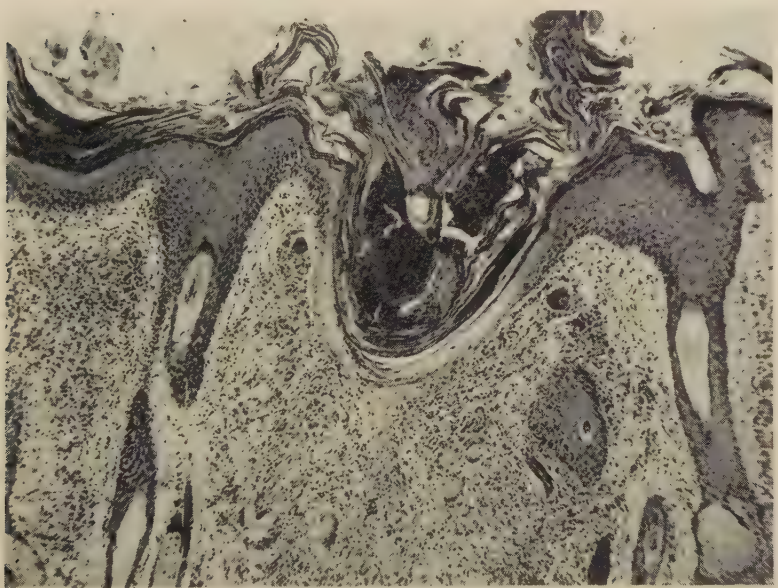


FIG. 10. An experimental bovine infection of the skin induced by scarification. Compare with Fig. 8. × 70.

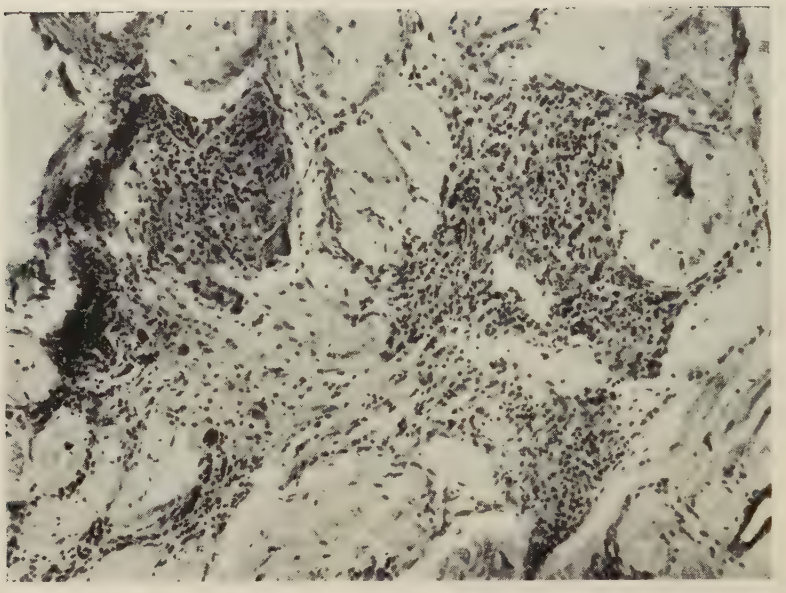


FIG. 11. Isolated small dermal lesion, at a distance from any major lesion. These are frequently seen in the perivascular connective tissue deep in apparently normal dermis. × 120.

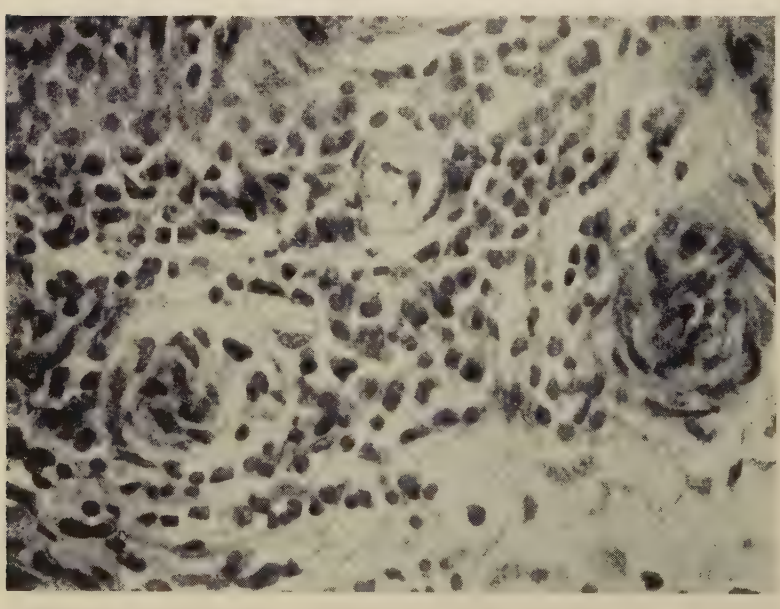


FIG. 12. Obliterative changes in the very numerous arterioles of a type 1 lesion. × 500.

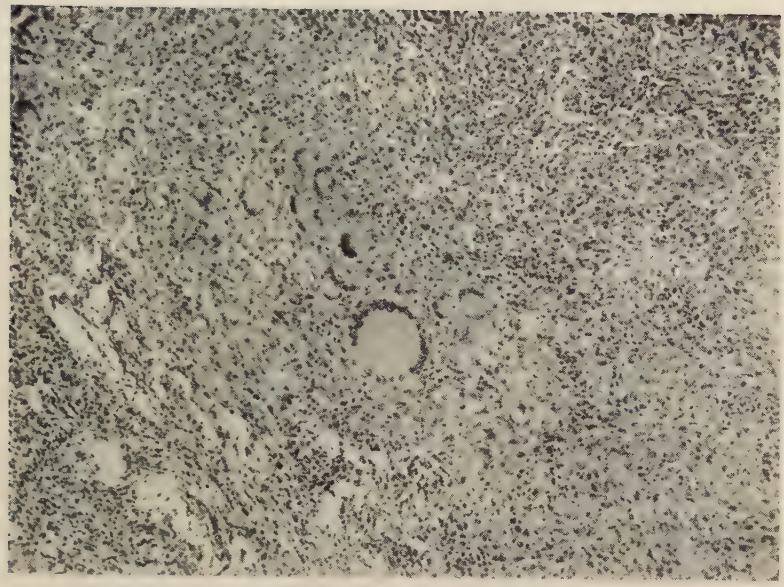


FIG. 13. Typical reaction of the dermis seen in skin lesion type 1. Very numerous giant cells. × 120.

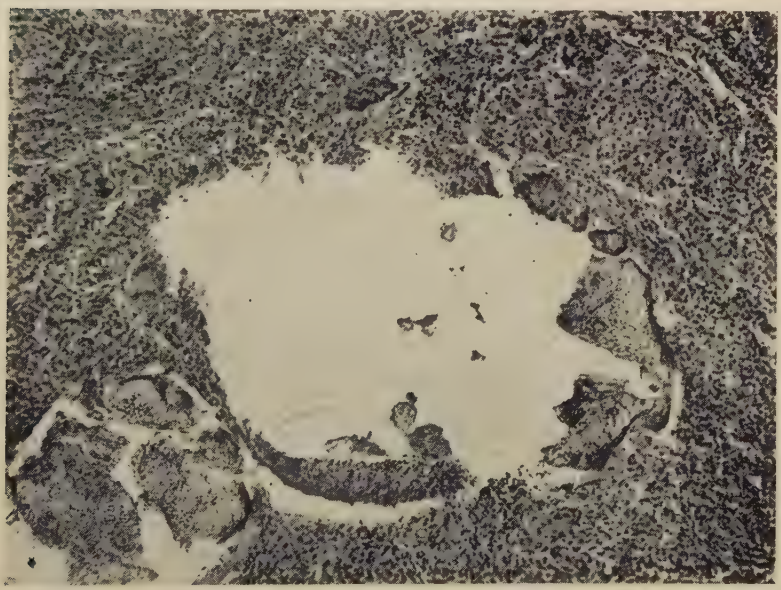


FIG. 14. Type 2 skin lesion. The major part of the necrotic debris has fallen out in sectioning, but some remnants can be seen at the margins. × 120.

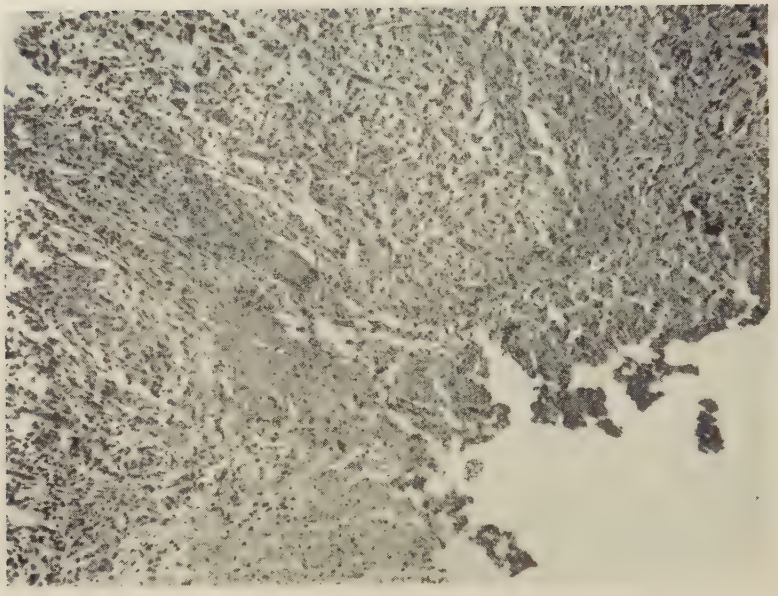


FIG. 15. A type 3 lesion margin. The fluid contents have escaped from the space but some portions still adhere. × 120.

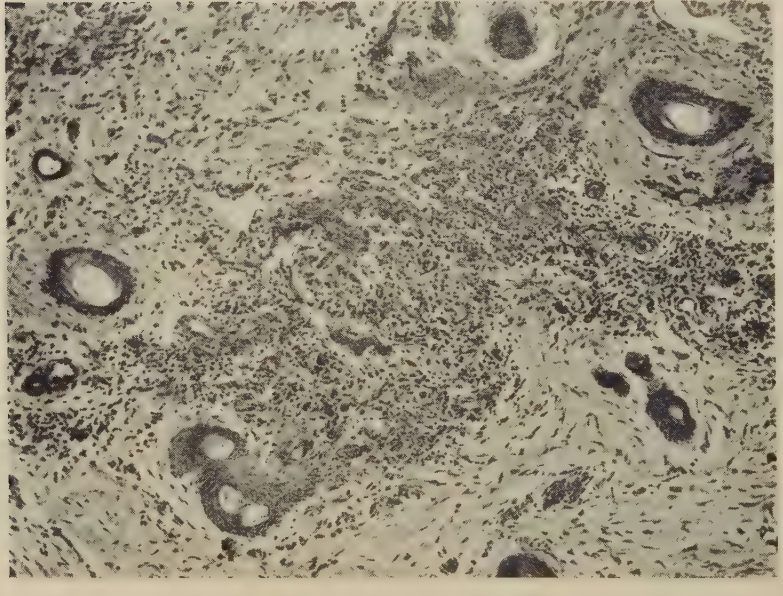


FIG. 16. The remnants of the re-absorbed lesions of Case 17. × 120.

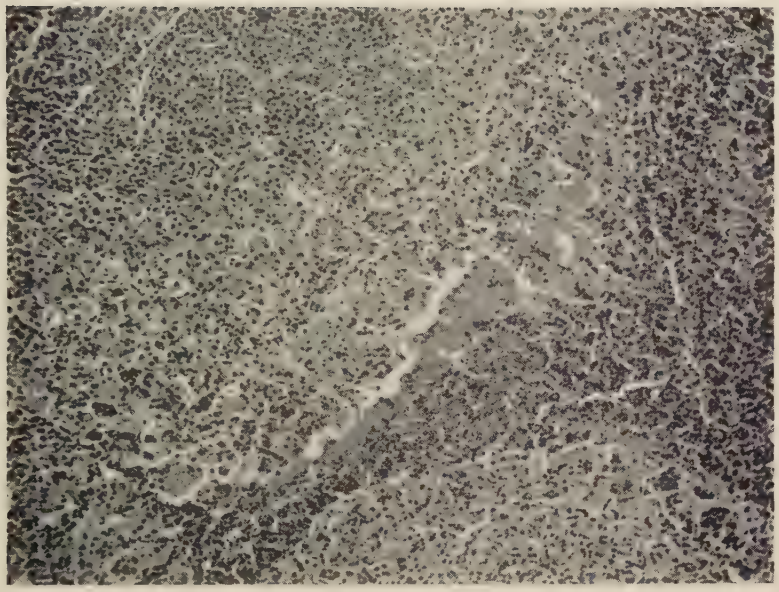


FIG. 17. A natural infection of the skin with bovine tubercle. The exudate seen stains with basic dyes and appears to be mucoid degeneration. The dark staining masses are granular leucocytes. × 120.

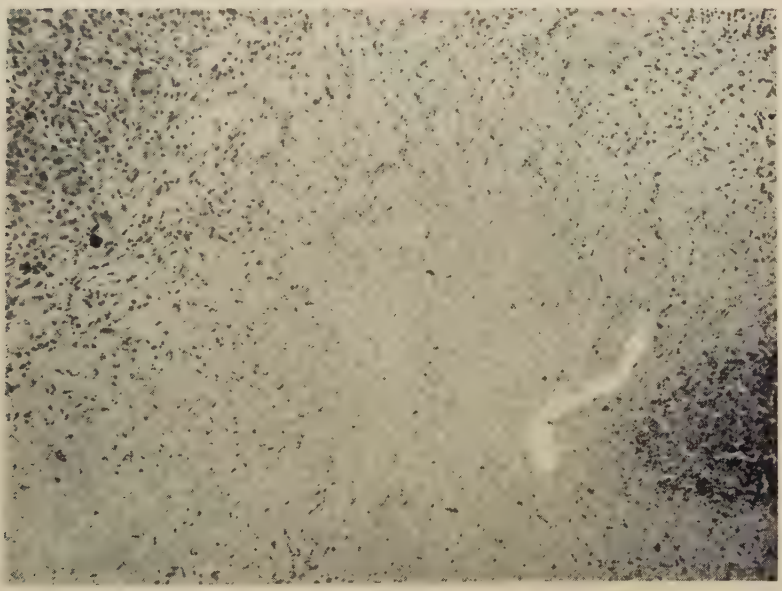


FIG. 18. An experimental intradermal bovine infection. Very extensive necrosis and a nest of granular leucocytes on the left. × 120.

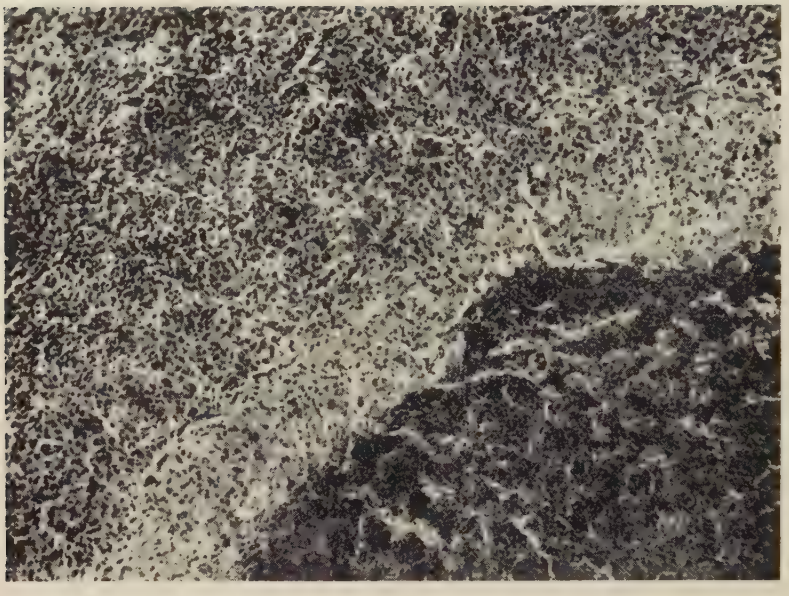


FIG. 19. An experimental avian infection. Only small areas of necrosis but a very massive invasion by granular leucocytes, the dark mass. × 120.

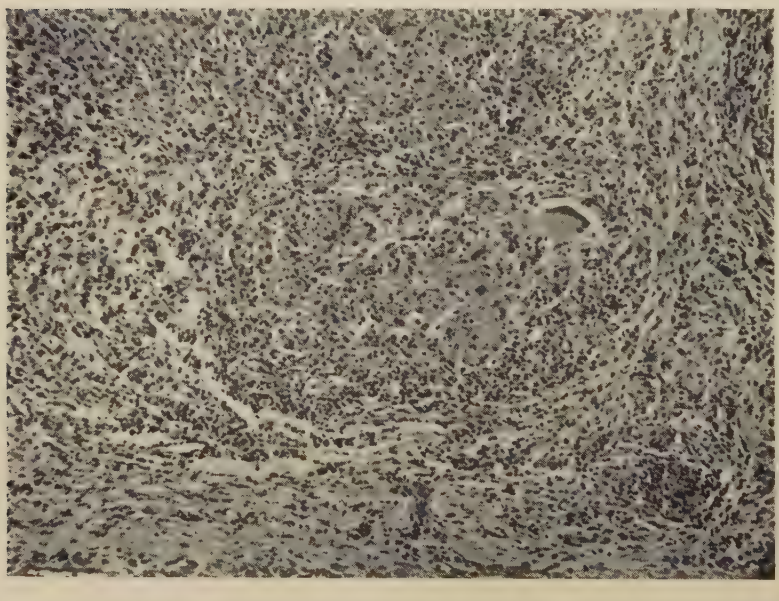


FIG. 20. A lesion in the dermis produced by inoculating *M. phlei* in liquid paraffin. × 120.

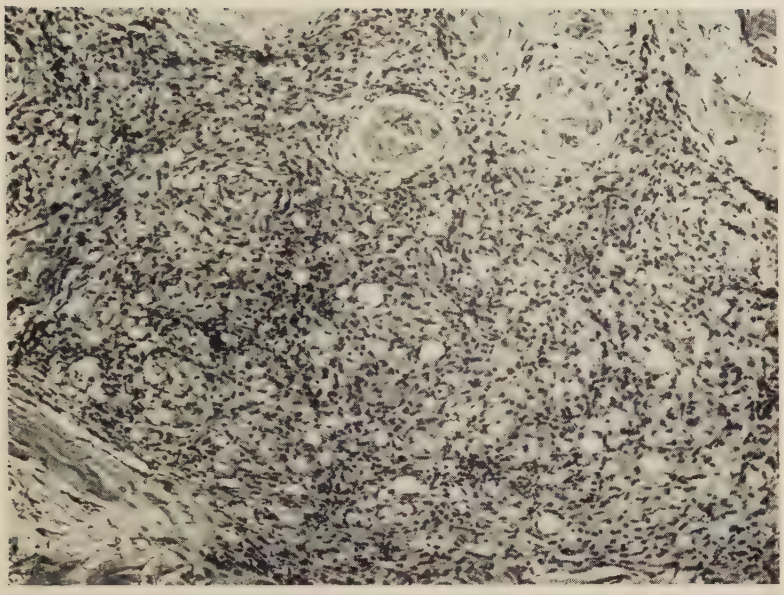


FIG. 21. The reaction of the skin to sterile liquid paraffin. The spaces left by dissolved droplets of paraffin are clearly seen. × 120.

FIG. 22. The reaction of a lymph gland to the intradermal inoculation of *M. phlei* in liquid paraffin. × 70.

FIG. 23. The gland lesion of Case 30, on the margin of the necrotic mass that has dropped out in preparation.

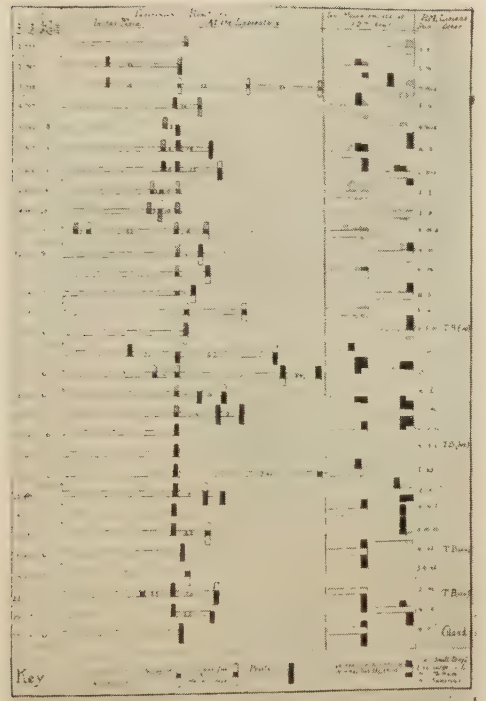


Figure 25

large percentage of cases. Mohler[15] reports that such fowls do not react to tuberculin and that no other lesions are found at *post-mortem*. Attempts to grow the organisms and to infect experimental animals were made, but " no evidence was obtained to prove that the organism was that of avian tuberculosis." Bunyea[16] records success in inoculating bumble-foot material in one fowl out of six. Affected fowls did not react to the tuberculin test except that one gave a doubtful reaction. Attempts to culture the organism were uniformly negative. Taking into consideration the position of the lesions of bumble-foot and of " skin-lesions " it would appear that there is a distinct possibility of the acid-fast organisms present in these two conditions being identical or related.

Summary.

1. A detailed description of the " skin lesions " of cattle, as seen by us, is given.

2. The reaction of these animals to avian and to bovine tuberculins is examined.

3. The geographical distribution of the cases we have received is shown.

4. Failure to reproduce the condition experimentally, and failure of cultural and biological examinations with skin-lesion material is recorded.

5. Although it seems very probable that these skin lesions are associated with the tuberculin reactions, there is no proof either that the skin lesions are able to provoke a response to tuberculin, or that the acid-fast organisms described are the cause of the sensitisation or even of the development of the skin lesions.

Addendum.

Since this report was composed we have obtained a pure growth of an acid-fast organism on Löwenstein's medium inoculated with material from the affected prescapular lymph gland of Case 30. It was only after four months incubation that some pin-point moist-looking colonies were seen on the surface, which on smear examination proved to be short acid-fast rods. Sub-cultures showed no evidence of growth after one month's incubation.

It is not possible at present to credit this organism with any etiological significance; its method of growth and the fact that guinea-pigs and fowls inoculated with the material from which the culture was obtained developed neither lesions nor sensitivity to avian or bovine tuberculins suggest that the organism does not belong to the tuberculosis group.

Acknowledgement.

We are indebted to Mr. G. J. Christie, of this laboratory, for certain *post-mortem* reports and small animal inoculations.

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Appendix.

(The histories of Cases 1-12 are recorded in the Preliminary Report[1].)
The increases in skin thickness at the 72nd hour only are recorded.

Unless otherwise stated, biological examination of groups of lymphatic glands was carried out, in every case, with negative results.

Case 13.

Heifer 841 which had never reacted in the field to the tuberculin test. In five tests the maximum skin increase recorded was 1/8. The two subsequent tests showed increases of 3/8 and 2/10 respectively, but the result was negative. Tested at the laboratory, however, a positive reaction was obtained to bovine tuberculin (increase of 9/7) and a negative to avian tuberculin.

No lesions were found at *post-mortem* examination except a number of skin lesions on the outer aspect of the right fore limb, in smears from which acid-fast organisms were demonstrated. Cultural and biological examinations of this material were negative.

Histologically typical lesions type 1 with external ulceration.

Case 14.

Heifer 845. In the field four negative tests were followed by a doubtful reaction to bovine tuberculin (increase 6/8). Two subsequent field tests were negative, and no reaction was obtained to either avian or bovine tuberculins at two laboratory tests.

At *post-mortem* no lesions were seen except one pea-sized skin lesion behind the left carpus, in smears from which no acid-fast organisms were found. Biological examinations of this material were negative.

Histologically a skin lesion type 2.

Case 15.

Cow 886, Shorthorn or Cross in good condition. No field tuberculin tests. At the laboratory gave a positive reaction to bovine with an increase of 14/6 with œdema. Did not react to avian tuberculin. This animal also

tested with "Phlein," a parallel preparation to tuberculin made from *M. phlei*. It did not react.

A chain of 25 lesions along the usual line of the right fore, chiefly on the antero-lateral aspect of the elbow, smears from which contained acid-fast organisms. Biological tests with this material negative. Histologically skin-lesions of type 2, the abscess contents being dry and gritty to the knife.

(A mediastinal lymph gland showed lesions which were proved by inoculation to be bovine tuberculosis. There were also two encapsulated lesions of the prescapular lymph gland on the right side in which acid-fast organisms were present; these unfortunately were not inoculated, it being assumed an extension from the mediastinal gland, quite unjustifiably as it equally well might have been connected with the skin lesions.)

Case 16.

Cow 917, Shorthorn, gave a positive reaction to bovine tuberculin, and a skin increase of 8/7. One month later it reacted to avian tuberculin, skin increase 21/7. Tested at the laboratory it gave a negative reaction to bovine tuberculin, a positive to avian (10/6) and a positive to Johnin (10/7).

No lesions were found at *post-mortem* examination except in and under the skin. The left fore leg showed two walnut-sized lesions below the carpus, of type 2 or 3, and there was a much thickened area of skin bearing the star-like scar shown in Fig. 5. This proved to have the histology of a type 1 skin lesion. Smears made from both types of lesion showed scanty acid-fast organisms. Cultural and biological tests proved negative.

Case 17.

Heifer 881. This animal gave six negative reactions to bovine tuberculin and then two doubtful reactions with a maximum thickening of 7/8. Caudal fold and vulva tests were negative. The animal was purchased for an abortion experiment, and, examined on arrival, showed four nodular skin lesions about the knee and fetlock of the left fore leg, and one lesion behind the knee on the right fore leg. It was seven months before the animal was available for *post-mortem* examination, during which time the lesions regressed until nothing could be seen or felt just before death. Meanwhile two tuberculin tests had been carried out, and in both instances (the last when there were no visible lesions) a positive reaction to bovine tuberculin was obtained (8/6 and 8/6). At both tests the avian result was negative, with a maximum increase of 4/6.

No lesions of any kind were seen at *post-mortem* except that careful sectioning of the skin in the region known to have had nodules showed a slight thickening of the dermis and a very small light brown focus that proved histologically to be a type 1 skin nodule. Biological tests were not made with this material.

Case 18.

Cow 941. After passing five tuberculin tests this animal gave a doubtful reaction to bovine tuberculin with a skin increase of 6/9. Tested at the laboratory on two occasions it reacted each time to avian tuberculin, maximum skin thickening 7/7, but failed to react to bovine.

No lesions of any kind were found except those in the skin. Two large lesions, the size of pullet's eggs, were present on the outside of the left knee, tense and containing fluid, and several smaller lesions were present close by. First noticed six months ago, when the size of damson plums. No acid-fasts were found in these lesions, and cultural and biological tests were negative.

Histologically the lesions fell into type 3 skin lesions, but the amount of granulomatous tissue present was very small.

Case 19.

Cow 929A, a Jersey, had passed ten tuberculin tests and then gave a doubtful reaction to bovine tuberculin with a skin reading of 5·5/4. Retested twice at the laboratory it reacted on each occasion to both bovine and avian tuberculin, the maximum skin reading in each case being 8/4.

With the exception of one skin lesion, no abnormalities were found at *post-mortem* examination. This nodule was the size of a walnut and lying on the inner aspect of the right carpus. Numerous acid-fasts were found in smears, but cultural and biological tests with the material again yielded negative results. Histologically the lesion was a mixed type 1 and type 2.

Case 20.

Cow 965 had passed 16 tuberculin tests in the field. It then gave a positive reaction to bovine tuberculin, with skin increase of 9/7.

Post-mortem examination showed nothing except a number of lesions up to walnut size on the outside of the right carpus in the dermis and subcutaneous tissues. There was some external ulceration. Fibrous capsules enclosed yellow semi-fluid material which contained numerous acid-fast organisms, and the dermal lesions were formed of typical light-brown tissue. Biological tests made from this material were negative. Histologically the lesions were of type 1 and type 3.

Case 21.

Cow 969. This animal gave a very marked reaction to bovine tuberculin. A number of lesions were present on the left fore leg following the usual knee to shoulder line. The larger nodules, about the size of walnuts, contained fluid, the smaller nodules dry crumbling necrotic material; the walls of both types of lesion had the typical light brown tissue well marked, and smears showed numerous acid-fast organisms, which however stained badly and seemed to be degenerating or dead. Biological and cultural tests with this material gave negative results.

(There were also present lesions in a mediastinal gland in which bovine tubercle were shown to be present by animal inoculation tests.)

Case 22.

Cow 970. Passed ten tuberculin tests and then reacted to bovine tuberculin with a skin increase of 6·5/4. Tested at the laboratory no reaction was elicited by bovine or avian tuberculin.

Only one lesion of any kind was found in this animal, and that was embedded in the dermis in front of the shoulder. It was the size of a small pea, and composed of a capsule enclosing yellowish rather fatty contents, smears from which showed a few acid-fast but darkly-staining pleomorphic organisms. No inoculations or cultures were made from this lesion, but histologically the walls had the typical structure of type 1 skin lesions.

Case 23.

Cow 981. Passed a number of tuberculin tests and then reacted to bovine tuberculin with skin increase of 11/7 with œdema. Retested at the laboratory it was negative to bovine tuberculin but reacted twice to avian tuberculin within seven days (skin increases of 7/6 and 6/6). Skin lesions were only found *post-mortem*, and consisted of two pea-sized nodules on the lateral aspect of the left fore leg just below the carpus. They were subcutaneous

in position and were of type 2, the soft yellow centres containing scanty acid-fast organisms. The prescapular gland of the same side appeared enlarged and showed hæmorrhages on the cut surface. No other lesions of any kind were found. The skin lesion material from this case was not tested culturally or biologically.

Case 24.

Cow 982. After passing a number of tests this animal reacted to bovine tuberculin, skin increase 10/7 with œdema.

A chain of skin lesions was present following the usual line, on the right fore leg. (Fig. 2.) There was a thick glairy discharge from one lesion that had pointed just above the carpus. The remaining lesions were subcutaneous and in the form of walnut-sized nodules and flattened masses of tissue up to 4 in. square. Histologically the flattened lesions were of type 1 and the nodular lesion of type 2. Acid-fast organisms were demonstrated in the former material only.

The prescapular glands of both sides seemed abnormally large and showed haemorrhages on the cut surfaces. Six small ulcers about 0·5 cm. diameter were found in the mucosa of the duodenum, but histologically these bore no resemblance to tuberculosis.

There were no other visible lesions.

Biological and cultural tests were not made.

Case 25.

Cow 987. Tested in the field with three different brands of bovine tuberculin by simultaneous injection at different sites in the neck, reacted to all three with the following skin increases: 7/7, 13/8 and 19/9, all with œdema.

Retested at the laboratory 22 days later, no response was obtained to either avian or bovine tuberculin.

When first seen there was a soft swelling the size of an orange just above and lateral to the left hock and several smaller lesions in the immediate vicinity. Fluid was removed *ante-mortem* from the large lesion and found to contain acid-fast organisms. *Post-mortem* and histological examination of these lesions showed them skin lesions, type 3. There was also a lesion of type 1 in front of the fetlock of the fore leg of the same side. The popliteal lymph gland of the affected leg was the size of a golf ball, firm and homogeneous on section.

Small pin-head and millet seed lesions were found in the mesenteric lymph glands and on one bronchial lymph gland, very suspicious of tuberculosis. There was also present a severe non-tuberculous mastitis.

Cultural and biological tests with the acid-fast containing fluid withdrawn *ante-mortem* were entirely negative.

Cultural and biological tests with the mesenteric and bronchial gland lesions failed to show evidence of tuberculosis.

Case 26.

Cow 993. This beast, a Red Devon, was not tuberculin tested in the field, but reacted at the laboratory to both avian and bovine tuberculin with skin increases of 11/6 and 8/6 respectively.

A very large number of lesions were present on both fore legs (Fig. 1), following the usual line from carpus to shoulder. The lesions were all subcutaneous in position and no dermal involvement was observed. At different sites characteristic specimens of type 1, type 2, and type 3 skin lesions were obtained, and acid-fast organisms were demonstrated in smears

In this animal the lesions had been noted to originate at the lowest point, and subsequently further lesions developed in the centripetal direction. We were not able however to demonstrate macroscopic or microscopic distinctions between the older and younger lesions.

Cultural and biological tests with skin material were negative.

(Small lesions were present in the mesenteric glands and were shown to be due to avian tubercle by both cultural and biological tests.)

Case 27.

Cow 975 was tested at least twice in the field and once at the laboratory. No reaction was obtained at any test to either avian or bovine tuberculins.

This animal showed four lesions behind and below the fetlock of the right hind leg. When first seen one lesion was the size of a tangerine, soft and with fluid contents. It was lanced and the creamy pus discharged brought to the laboratory for examination. Smears from this pus showed a very large number of acid-fast organisms, arranged in clumps or colonies (Fig. 7). Cultural and biological tests with this material were negative.

No lesions other than the skin lesions were found at *post-mortem*. The skin lesions were of type 3, with considerable skin involvement where pointing had occurred. The popliteal lymph gland of the affected side was the size of a small plum, and had a rather " marbled " appearance on section.

Case 28.

Heifer 2. This animal gave a doubtful and later a positive reaction to bovine tuberculin in the field. Twenty-six days later a negative reaction to bovine tuberculin and a positive reaction to avian tuberculin (skin increase 16/6) was obtained at the laboratory.

Two lesions, walnut-sized, were present on the left fore leg. There was no skin involvement, subcutaneous in position, and one proved to be of type 1 and the other of type 2. Acid-fast organisms were demonstrated in smears. Cultural and biological tests with this material were negative. (There were also present typical avian lesions in two mesenteric glands. Biological tests with this material were not conclusive, some of the guinea-pigs and fowls have reacted slightly to avian tuberculin, but no lesions were found on *post-mortem*.)

Case 29.

Heifer 3 reacted to bovine tuberculin in the field (skin increase 5/6 with œdema) at its first test. Retested at the laboratory 25 days later no reaction was obtained with bovine tuberculin, but a positive was given to avian tuberculin (skin increase 10/6).

Four small lesions were present at *post-mortem* on the L.H.S. neck about the site of a tuberculin test. There was no involvement of the skin and the lesions were of type 2.

Cultural and biological tests with this material were completely negative. No other lesions were found.

Case 30.

Cow 4. An aged animal that had never been tuberculin tested until receipt at the laboratory, when it reacted to both avian (10/6) and bovine (6/6) tuberculin.

A chain of skin lesions followed the usual line up the left fore leg. When at *post-mortem* the shoulder lesions were traced they were found to dip into the superficial muscles and the last one lay against the prescapular gland. The gland itself was enlarged and two firm centres could be felt in its substance. On section these were found to be foci of gritty necrosis.

Very scanty acid-fast organisms were demonstrated in smears from both skin lesions and gland. The skin lesions were of type 2, with dry gritty centres.

Histologically the gland lesions were not really typical of tuberculosis; they are further considered under the histology section.

Cultural and biological tests with the gland material have proved completely negative. The test animals did not react to bovine or avian tuberculins.

A calculus was present in one kidney pelvis, but otherwise no lesions were found.

The following two cases are interesting specimens that have been received at the laboratory for diagnosis.

Case 31.

Portions of muscle removed from the shoulder of an animal that had reacted to bovine tuberculin. In the muscle tissue, but separated from it by fibrosis, were several skin lesions type 2, the largest being walnut size. The field report stated that the smaller lesions were not seen until the trapezius muscle had been removed. The lesions were quite independent of the skin. Smears from the necrotic contents of these lesions showed a few acid-fast bacilli. Sections showed the usually histological structure of type 2 skin lesions, the fibrous tissue of the lesion wall appearing to be an extension of the interfascicular tissue of the muscle. Biological tests with this material were negative.

Case 32.

Material taken from a Friesian cow that had passed three tuberculin tests and then reacted twice in 15 days to bovine tuberculin. The skin material showed very extensive lesions of skin tuberculosis type 2. The distal extremity of a fore limb is shown in Fig. 4, where it will be noted the lesions have formed a confluent nodular mass at the sides and back of the first phalanx. Scanty acid-fast organisms were found in smears. The lesions had first been noted at least three years ago, and after reaching walnut size the nodules increased in number but not in size.

Biological tests with this material were not made.

This is the only case of lesions below the knee in a fore limb that we have seen.

A number of other specimens received have been diagnosed as skin lesions, but the data were not of sufficient interest to warrant publication.

THE CEREBROSPINAL FLUID IN THE BOVINE: ITS COMPOSITION AND PROPERTIES IN HEALTH AND DISEASE, WITH SPECIAL REFERENCE TO "TURNING SICKNESS."

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The literature on the cerebrospinal fluid in the bovine appears to be extremely scanty, and such data as has been published deals only with its general physical properties, cell count, protein, glucose, chlorides, and in one instance calcium content. The earliest reference appears to be that of Nawratzki (1897), and since then only two others can be traced: Egli (1936) and Fedotov (1937).

This investigation was commenced primarily in connection with research being carried out on "Turning Sickness"—an encephalitis of cattle in Uganda—the pathology of which has already been described in detail by Mettam and Carmichael (1936). Owing to lack of data concerning the normal bovine cerebrospinal fluid, it was decided to undertake the analysis of normal fluids for comparison with those from turning sickness and other cases.

Technique.

Technique for the withdrawal of Cerebrospinal Fluid in Bovines.

The technique for the withdrawal of C.S.F. from cattle given by Egli (1936) is similar in many respects to that which we have used for some years. A detailed account, however, is given here.

The area selected is the atlanto-occipital space. The animal is secured in the lateral decubitus position, and the head flexed to its full extent. The surface markings are the lateral edges of the wings of the atlas, which should be followed round until the anterior border is reached. A line joining the anterior borders across the neck indicates the plane insertion in which the needle should be inserted in the middle line. The hair is shaved from the area and the skin cleaned with spirit. The size of the animal determines the type of needle required. In full grown native cattle a needle measuring 2·5 inches, with a bore of 1/16th inch, attached to a 50 c.c. syringe is used, and the requisite fluid aspirated. In calves, an ordinary No. 1 hypodermic needle can be used, but care is necessary in this case to avoid bending and possible breakage. The fluid is aspirated into a 10 c.c. syringe. The advantages of this method are the comparative ease with which a non-contaminated specimen can be obtained and the smallness of the trauma, thus avoiding excessive damage to the membranes with resultant blood admixture in the sample.

Cytological Examination and Protein.

The cell count was made with a Neubauer Hæmocytometer, the fluid being diluted with one part of 3 per cent. acetic acid to 19 parts of C.S.F. In a few cases the *p*H of the fluid was determined colorimetrically, after diluting 1 : 10 with distilled water. The test adopted for globulin was the well-known Nonne-Apelt reaction with ammonium sulphate, the presence and intensity of the ring formed at the junction of the liquids being taken as the criterion for increased globulin content. Various other tests for globulin were also tried out, including Pandy's phenol method and the recently described tannic acid method of Newman (1938), but the latter was found to give a considerable number of false positives with fluids from known healthy animals.

The total protein was estimated by the diaphanometric method of Mestrezat (1913), using protein standards prepared from blood serum after careful determination of the total nitrogen by the Kjeldahl method. The range of standards employed is from 0·005 per cent. to 0·05 per cent. protein ; the cerebrospinal fluid being diluted with normal saline if higher values were obtained.

Colloidal Gold Reaction.

Lange's colloidal gold reaction was carried out by the method of Patterson (1931), using an oxalate-reduced gold sol prepared from the double salt sodium gold chloride. The standardisation of the gold sol with fresh oxyhæmoglobin was controlled by determination of the sensitive *p*H range estimated colorimetrically by the freezing and thawing method of Pennycuick, Woolcock and Cowan (1938).

It was unfortunate that most of the severe turning sickness cases occurred before the colloidal gold test and total protein standards had been set up, but it is hoped that further cases will be available for these, now that they have been established on a permanent basis.

Chemical Analysis.

The chemical analysis was performed on protein-free filtrates by the colorimetric methods of Folin and Wu for glucose, non-protein nitrogen and creatinine ; the filtrate being prepared with 1 volume of C.S.F., $8\frac{1}{2}$ volumes of water, and a $\frac{1}{4}$ volume each of 10 per cent. sodium tungstate and $\frac{2}{3}$ normal sulphuric acid. The urea nitrogen was determined by the method of Karr (1924), and chlorides by that of Whitehorn (1921) on the same filtrate. Calcium was estimated by the original method of Kramer and Tisdall (1921) using 2 c.c. of C.S.F. No estimations of phosphorus or uric acid in the cerebrospinal fluid have so far been carried out.

Normal Bovine C.S.F.

The cerebrospinal fluid of the normal bovine should be a water-clear, transparent, colourless fluid in which no clot is formed after standing. The results obtained from the examination of 33 specimens of cerebrospinal fluid taken from known healthy bovines comprising 28 bulls, four cows and one calf, are summarised in Table I.

TABLE I. NORMAL BOVINE CEREBRO-SPINAL FLUID

	GLOBULIN (Nonne-Apelt)	LEUCOCYTES (per cu.mm)	TOTAL PROTEIN (mg./100cc)	CHEMICAL ANALYSIS: GLUCOSE	CHLORIDES (as NaCl)	N.P.N. (Mg. per 100cc.)	UREA N.	CREATININE	CALCIUM	LANGE TEST.
AVERAGE OF 33 SPECIMENS EXAMINED :-	Negative.	10.6	34.3	36.8	685.1	16.1	10.8	1.4	5.5	Negative
N.B. The number appearing under each average denotes the number of tests carried out for each constituent.	(25 = neg; 4 = Weak Plus to Plus; 4 = Doubtful owing to presence of Blood.)	(21)	(23)	(22)	(20)	(22)	(17)	(20)	(8)	(17)
MINIMUM:-		1	20	21.5	643.5	10.4	8.8	1.26	4.3	
MAXIMUM:-		36 (Only 4 above 15)	60 (Only 5 above 40)	53.2 (Only 4 below 30)	734.3	40.3 (Only 1 above 21.8)	15.2	1.54	6.9	
BOVINE REFERENCES										
NAWRATZKI (1897) :-	-	-	16 - 28	46	700	-	-	-	-	-
EGLI (1936) :-	Negative.	(Low)	-	-	-	-	-	-	-	-
FEDOTOV (1937) :- (22 cows; 4 oxen; 6 calves.)	Negative.	1 - 6	11.5-20	54 - 60	620 - 760	-	-	-	5.2 - 6.1	-
HUMAN REFERENCES FOR COMPARISON :-	Negative.	1 - 6	14 - 50	45 - 100	700 - 750	15-35	6-25	0.7-2.0	5.0 (4.4 - 6.6)	Negative.

N.B. The above figures for HUMAN C.S.F. have been compiled from the following sources:-

BEAUMONT & DODDS (1931); STEWART & DUNLOP (1937); STITT, CLOUGH & CLOUGH (1938); ANDERSON (1935).

Excluding a few specimens showing slight blood contamination, the *leucocytes* appear to vary from 1 to 18 per cu. mm., a count of 7 to 12 per cu. mm., being frequently obtained. The normal bovine cerebrospinal fluid thus appears to have a slightly higher cell count than the human cerebrospinal fluid.

Globulin shows no increase except where there is blood contamination, and *Lange's colloidal gold* test is consistently negative in the normal fluid.

The *Total Protein* average of 34·3 mg. per cent., with a probable range of 20 to 40 mg. per cent., is very similar to that generally accepted for human cerebrospinal fluid (average 30 mg. per cent.), though somewhat higher than that given by Fedotov (1937) and Nawratzki (1897) for the bovine. A difference may exist between European and African breeds in this respect, parallel to that observed by various workers in the erythrocyte concentration of bovine blood.

The average *Glucose* content of 36·8 mg. per 100 c.c., with a probable range of 30 to 53 mg. per 100 c.c., is slightly lower than Fedotov's (1937), the difference probably being due to the method of estimation. The average obtained corresponds to 75 per cent. of the blood glucose normal (50 mg. per 100 c.c.), as is the case in man.

The range for *chlorides* is slightly lower than that found in human cerebrospinal fluid, and approximates to the values quoted for the bovine by Nawratzki (1897) and Fedotov (1937).

The normals obtained for *non-protein nitrogen* and *urea nitrogen* bear approximately the same relation to those of the blood as in man, while the *creatinine* level is also the same as that in the blood. The ratio of urea nitrogen to non-protein nitrogen, however, seems considerably higher in bovine cerebrospinal fluid (70 per cent.) than that generally stated for human cerebrospinal fluid (40 to 50 per cent.).

The *calcium* content corresponds to that quoted by Fedotov (1937), and is about 50 per cent. of the serum calcium as stated by Anderson (1933), Gernez and Lespagnol (1931), and Cohn, Kaplan and Levinson (1939) for human cerebrospinal fluid.

We have thus established the normal characteristics and chemical constituents of bovine cerebrospinal fluid (with the exception of phosphorus and uric acid), and will now consider any abnormalities encountered in disease.

Bovine C.S.F. in "Turning Sickness."

Fifteen specimens of cerebrospinal fluid from cases of turning sickness are considered in this paper, all of which had shown nervous symptoms at one stage or another. The fluid in these cases was often under considerable pressure and bubbled out into the barrel of the syringe before any suction was applied.

The fluid was frequently turbid, and in many cases amber coloured. The chief characteristics comprised a high cell count, increased globulin and total protein, and in some cases the formation of the well known pathological "cobweb" type of clot on standing. The results obtained are given in detail in Table II.

TABLE II. BOVINE CEREBRO-SPINAL FLUID IN "TURNING SICKNESS"

DATE.	ANIMAL NO:-	CLINICAL NOTES:-	APPEARANCE of C.S.F:-	GLOBULIN. (Nonne-Apelt)	LEUCO-CYTES (per cu. mm.)	TOTAL PRO-TEIN. (mg.%)	CHEMICAL ANALYSIS (Mg. per 100cc.) GLU-COSE	CHLORIDES (as NaCl)	N.P.N.	UREA N.	CREATI-NINE.	CAL-CIUM	LANGE'S COLLOIDAL GOLD TEST.
21-4-38	3079.	Acute.	Clear, pale amber, "cobweb" clot formed.	± Inc.	209	-	51.7	1098	10.71	-	2.0	-	- (Kahn Test ± doubtful)
3-5-38	3556.	Subacute.	Clear, no clot.	± Sl.Inc.	42	-	-	-	-	-	-	-	-
4-5-38	3557.	Subacute becoming chronic.	Turbid : blood present; no clot.	? V.Sl.Inc.	11	-	62.9	709.5	15.0	-	1.6	-	-
31-5-38	"	"	Blood clot & deposit.	± Inc. R	175	-	40.0	685.0	18.45	8.46	1.45	-	0001132100
21-7-38	"	"	Clear, no clot.	± V.Sl.Inc.	10	55	38.6	695.0	15.57	12.7	1.47	-	-
11-5-38	3462.	Subacute.	Turbid, blood, small clots.	± Sl.Inc.	72	-	32.5	635.5	15.0	-	1.49	-	-
27-8-38	K592.	Chronic.	Blood clot & deposit.	++ Inc. R	-	300	-	-	-	-	-	-	0000001000
"	K512.	"	Clear, no clot.	- Nil.	-	50	-	-	-	-	-	-	0011000000
7-10-38	K995.	Subacute.	Clear, "cobweb" clot.	± V.Sl.Inc.	21 Ø	100	-	-	-	-	-	-	3332210100
"	K996.	Chronic.	Blood deposit, fibrous clot.	± Inc. R	60 Ø	100	-	-	-	-	-	6.0	0000110000
"	K896.	"	Clear, no clot.	- Nil.	9 Ø	25	20.2	701.3	18.5	16.06	1.44	5.4	0000000000
"	K150.	"	Slightly turbid, trace blood, fibrous clot.	- Nil.	15 Ø	55	22.6	693.0	17.8	16.06	1.58	6.2	0000000000
"	K872.	"	Slightly turbid, trace blood, "bladder" clot.	++ Inc. R	18 Ø	400	-	-	-		-	6.9	0000000010
	EXPERIMENTAL:-												
25-4-38	3466.	Healthy.	Clear, no clot.	- Nil.	18	-	32.0	-	10.44	-	1.52	-	-
4-5-38	"	"	" " "	- Nil.	-	-	28.0	651.8	21.84	-	1.41	-	-
19-5-38	"	15 days after inocu-lation ex No. 3557.	Turbid, very pale straw colour, 'fluffy' clots formed.	± Inc.	558	-	31.2	618.8	22.7	-	1.27	-	0012521110
19-5-38	2911.	Healthy.	Clear, no clot.	- Nil.	9	-	-	-	-	-	-	-	0011110000
31-5-38	"	12 days after inocu-lation ex No. 3466.	Turbid, blood deposit, very pale amber, small "cobweb" clot.	± Inc.	190	-	21.0	668.0	19.23	-	1.35	-	0000221100

NOTES:- Under GLOBULIN : Inc.=Increase; Sl.=Slight; V.Sl.=Very slight; R = Reaction doubtful owing to presence of blood.
Under LEUCOCYTES: Ø = Cell count made on fluid after 24 hours.
Under LANGE'S COLLOIDAL GOLD TEST:
The results are expressed according to the generally accepted notation:-

Complete decolorisation	= 5	Lilac or Purple	= 2
Pale Blue	= 4	Bluish-Pink	= 1
Blue	= 3	Pink or Cherry Red	= 0

Complete blood analysis was also carried out on four turning sickness cases, but showed no abnormality other than a varying degree of anæmia.

Experimental Cases.

Numerous attempts at transmission of turning sickness have been carried out, but so far the typical symptoms have not been reproduced.

Bovine No. 3466.—This animal was inoculated intrathecally with 5 c.c. of cerebrospinal fluid ex No. 3557. It showed symptoms of lethargic encephalitis and died 15 days later. Both the inoculum and the cerebrospinal fluid of No. 3466 proved sterile on culture. On *post-mortem* examination there were no macroscopic lesions in the brain and sections showed congestion of the cranial capillaries but no perivascular infiltration.

Bovine No. 2911.—This animal received 5 c.c. of cerebrospinal fluid intrathecally from No. 3466. The fluid was drawn the day prior to death, and was sterile on culture. It showed a temperature reaction of 103° F., from the 8th to the 12th day following inoculation. Otherwise no symptoms were recorded and the animal was discharged two months later.

Summary.

It is felt that we have insufficient data on which to draw conclusions regarding any chemical changes in the cerebrospinal fluid in turning sickness, though the alteration in the ratio of urea nitrogen to non-protein nitrogen in the case of bovine No. 3557 suggests a disturbance in basal metabolism. (The urea nitrogen was 45·8 per cent. of the N.P.N. on 31.5.38, and 81·5 per cent. on 21.7.38).

The general characteristics of the cerebrospinal fluid in turning sickness have been described.

Bovine C.S.F. in Miscellaneous Diseases.

Five specimens of cerebrospinal fluid were examined from cases of rinderpest, trypanosomiasis and East Coast fever.

No abnormalities could be observed in the two rinderpest specimens other than a slight globulin increase in one case.

In the case infected with *T. vivax* a slight globulin and protein increase was noted, as occurred also in one of the two specimens from East Coast fever cases.

Conclusions.

(*a*) Thirty-three specimens of cerebrospinal fluid from known healthy bovines have been examined, and normal values established in regard to cell count, globulin, Lange's colloidal gold test, and chemical constituents.

(*b*) Fifteen specimens of cerebrospinal fluid from turning sickness cases were examined, in which the chief changes noted were increased

cell count globulin and total protein, and in one case a positive Lange reaction.

(*c*) Five specimens of cerebrospinal fluid taken from cases of rinderpest, trypanosomiasis and East Coast fever showed little abnormality.

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PRELIMINARY OBSERVATIONS ON THE EFFECTS OF A LOW CALCIUM DIET ON GOATS AND KIDS.

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As a result of experimental investigations on the effects of low or unbalanced mineral diets fed accidentally or intentionally to horses, cows and sheep, the influence of calcium and/or phosphorus deficiencies on domestic animals has been firmly established in many parts of the world. It is not intended in this note to review the literature on this matter, since the published works of Theiler and his co-workers give an adequate survey of the results of past investigations on this subject. Although spontaneous bone diseases in goats have been reported frequently (Christeller, 1924; Marek and Wellmann, 1931; Knaggs, 1926) we believe that little experimental work has been done on these animals, so that in that sense the results to be presented provide new data, particularly from the blood chemistry point of view, to show that kids are eminently suitable ruminants for work on diets of low calcifying properties and, in particular, of low calcium content.

The deficiency condition occurred accidentally in goats and their kids kept for general laboratory experimentation and not for nutritional work. During the summer and early autumn periods the animals were allowed free range and subsisted almost entirely on grass. In the winter and early spring, however, they were almost continuously confined to pens with concrete floors. The diet which they received comprised oats, bran and a little hay, *i.e.*, a ration very similar in composition to those reported by Marek and Wellmann to be responsible for calcium deficiency diseases in goats. It appears from extended observations that this spontaneous deficiency had a seasonal variation and this, probably for climatic and dietary reasons, showed a peak in spring. This recalls the incidence of rickets in children.

It was found that the animals failed to thrive on this diet: in particular, two of the breeding nannies (Nos. 97 and 98) lost condition rapidly during the winter period. One of these (97) showed an obvious enlargement of the facial bones, swollen knee and hock joints and malformation of the hooves. Eventually complete posterior paresis developed, the animal was unable to or disinclined to feed, and finally died. The symptoms indicated that both these goats were probably suffering from calcium deficiency. At the

suggestion of M. Mellanby blood samples were taken from both animals. The figures obtained on these samples were:—

No. of Goat.	*Ca mg/100 c.c.*	*Inorg. P. mg/100 c.c.*	*Phosphatase (King & Armstrong units).*
97 (died)	4·7	Too stained	195
98	6·9	3·58	436

The results were so striking that it was decided to run a small scale experiment, using five six-week-old kids and including the nanny (98) mentioned above. The purpose of this experiment was to establish that we were dealing with a calcium deficiency and to determine to what extent this condition could be counteracted by supplements of $CaCO_3$ or vitamin D provided separately and together when given (*a*) as preventives of calcium deficiency, and (*b*) when the depletion of calcium was at a sufficiently low level to threaten the life of the animals.

Calcium and Phosphorus Requirements of Goats.—We have been unable to find in the literature data on the actual calcium and phosphorus requirements of goats, but it is stated by Franklin (1934) that for a 50 lb. sheep consuming approximately 15 lb. of dry matter per week, the daily requirement of CaO is 5 to 7 grammes, and of P_2O_5 is 3·50 grammes, *i.e.*, a CaO/P_2O_5 of 1·43 : 1 to 2 : 1.

By comparison with these values it might be permissible to deduce that the requirements of a goat of 20 lb., eating 8 lb. per week, which might be regarded as an average figure, would be between a half and two-thirds of a sheep, that is, CaO 2·4 to 3·5 grammes, and P_2O_5 1·8 to 2·25 per day. It may be noted that Marek and Wellmann (1931) reported the experimental production of osteitis fibrosa in a year-old goat when the CaO in the ration was 2·38 grammes per day and when there was a relative excess of phosphorus.

Ration.—Five six-weeks-old goats were available for the feeding experiments. Three (106, 108 and 110) were placed on a diet which was very poor in calcium, while the remaining animals (107 and 109) were allowed a supplement of $CaCO_3$. With the exception of 110 which was born of goat 97, an animal which subsequently died in tetany, the kids selected for the experiment came from apparently normal mothers. All the kids received a basal ration composed of equal parts of flaked maize and bran and a minimum amount of hay. Flaked maize was used because, as shown by Woodman and Stewart (1927), it is extremely low in calcium, but is otherwise eminently suitable for feeding purposes on account of its high digestibility, moderate protein content and richness in carbohydrate. It is known that foodstuffs such as maize and bran and, in particular, hay, show appreciable variations of Ca and P content, so that in order to obtain precise data it would have been

better to have analysed each sample at the beginning of the experiment and at any time when fresh samples were added, but this was not done. There can be no doubt, however, that the diet had a low calcium content and relative to this a higher P content (Ca : P equals 1 : 1·2). A considerable amount of phosphorus must have been in the form of phytin. Samples of the ration were analysed in January, 1939. Total phosphorus was determined by Neumann's method, using the modification of McCance and Shipp (1933). Phytin phosphorus was estimated by the method of McCance and Widdowson (1935). The calcium was determined in the ash by the usual oxalate precipitation.

	Ca.	*CaO.*	*Total P.*	*Total P_2O_5*	*Phytin P.*
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Maize	0·019	0·026	0·190	0·435	0·103
Bran	0·029	0·128	0·507	1·161	0·146
Hay	0·277	0·387	0·110	0·253	Too small to estimate

The amounts of food eaten altered with the growth of the animals and in some cases varied with the appetites, more particularly in those receiving the deficiency basal ration only. The lowest CaO and P_2O_5 daily intakes in the food, when no supplement of $CaCO_3$ was given, were CaO, 1·09 and P_2O_5, 2·24, and the highest, CaO, 1·68 and P_2O_5, 3·66, from which it would appear that even if all the Ca were absorbable there was probably a true calcium deficiency. The effect of the low calcium intake would, as has been shown by Mellanby (1937) in the case of canine rickets, be accentuated by any other factors such as a lack or deficiency of vitamin D, the presence of phytic acid phosphorus or any abnormal Ca/P ratio. With regard to the vitamin D content of the food, no determinations were made but it can be stated that none of the constituents is rich in this vitamin. For example, the value of a New Zealand sun-cured hay is given as 6 I.U./100 grammes, which is about the same as the value which has been assigned to maize silage, *viz.*, 6·5 I.U./100 grammes (Fixsen). In all probability the hay used for these goats had no, or very little, vitamin D. Approximately one-third of the phosphorus was present as phytic acid phosphorus.

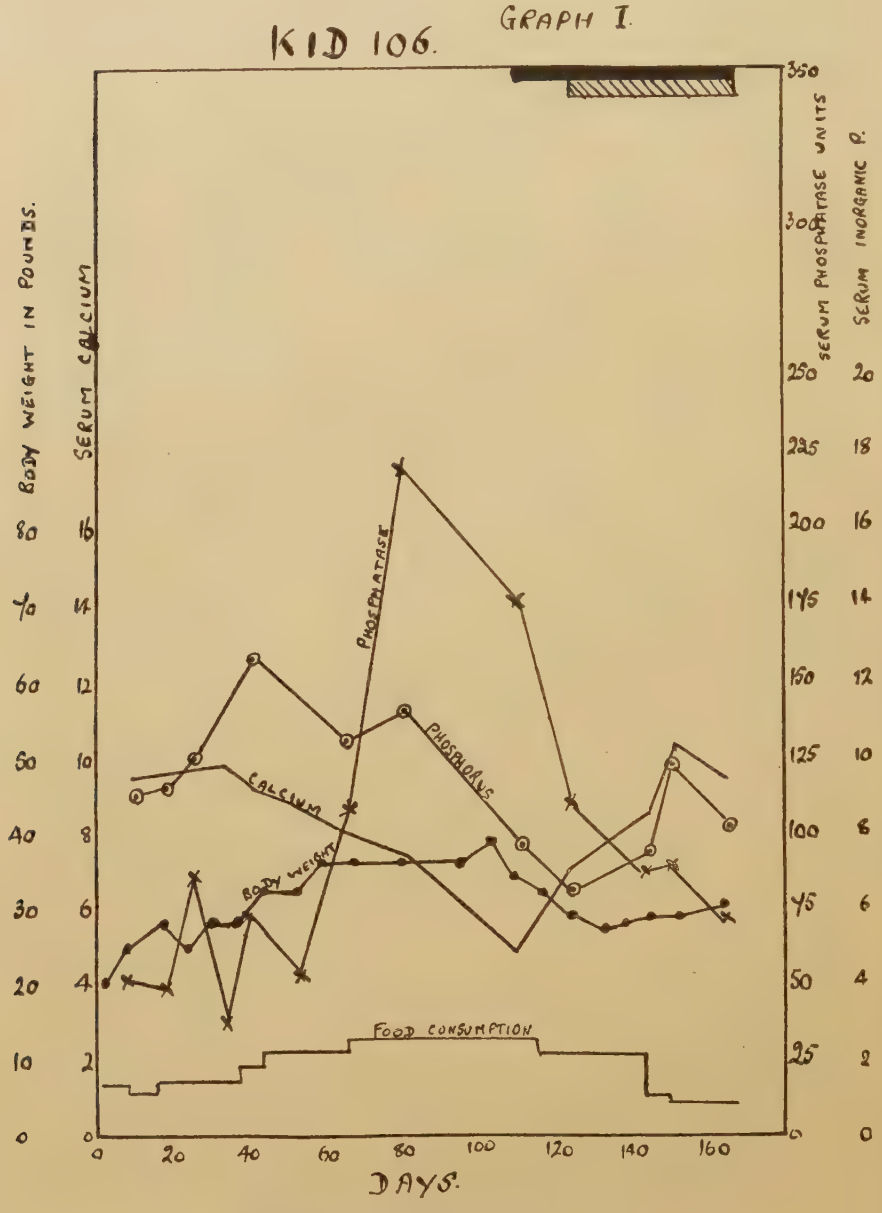
When calcium carbonate was given to the kids (107 and 109) it was mixed with the rest of the food, 8 grammes per day being given (equal to 4·48g. CaO). At certain periods during the experiments vitamin D was also given to some of the kids. It was provided in the form of Radiostol diluted with peanut oil and given directly. The dose was equivalent to that for a child, *viz.*, 2,000 I.U. per day.

Blood samples for serum analyses were taken at first weekly and later every fortnight. Serum calcium was determined by the method

of Collip and Clark (1925); inorganic phosphorus by the method of Fiske and Subbarow (1925) and serum phosphatase by the King and Armstrong (1934) method.

Treatment of Experimental Animals.

(*a*) *Animals on the Deficient Basal Ration.*—Kids 106, 108 and 110 were placed on the basal ration, but the diet for 108 was altered


KID 106.
GRAPH I.
BODY WEIGHT IN POUNDS.
SERUM CALCIUM
SERUM PHOSPHATASE UNITS
SERUM INORGANIC P.
PHOSPHATASE
PHOSPHORUS
CALCIUM
BODY WEIGHT
FOOD CONSUMPTION
DAYS.
0 20 40 60 80 100 120 140 160
0 2 4 6 8 10 12 14 16
0 10 20 30 40 50 60 70 80
0 25 50 75 100 125 150 175 200 225 250 300 350
0 2 4 6 8 10 12 14 16 18 20

Black strip indicates Calcium Carbonate,
Crossed strip indicates Vitamin D.

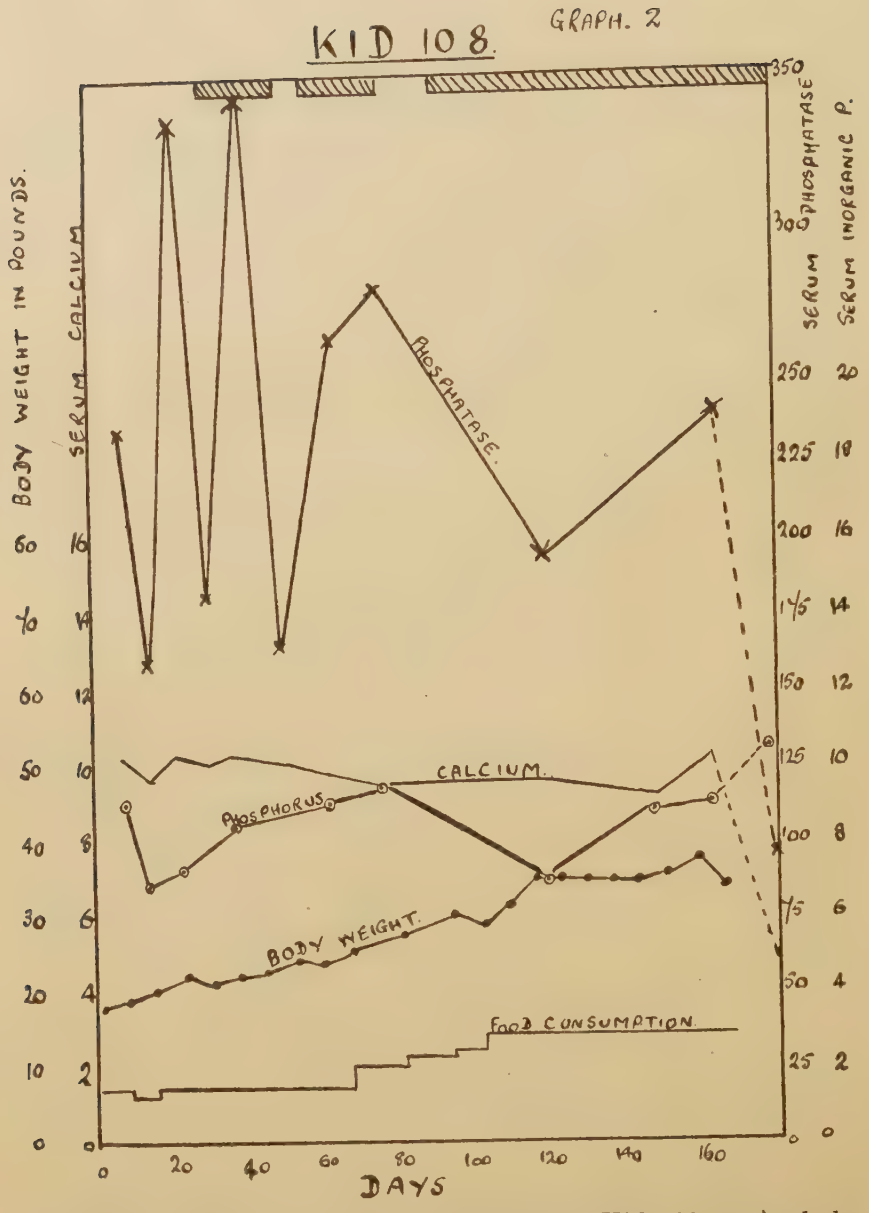
at a relatively early stage so that the results obtained with this animal can be more conveniently considered in the next section. Kid 110 showed a steady and rapid loss in weight and died with symptoms of tetany and a serum calcium of 5·0 milligrammes after one month on the diet. Kid 106, born of a normal mother, withstood the effect of the deficiencies of the basal ration rather better and even grew well at first, although the serum calcium fell gradually to about 8 milligrammes. After 14 weeks, however, it began to lose weight rapidly and became very ill with enlarged facial bones, nearly complete paresis and marked deformity of the feet. During this period the weight diminished from 38 pounds to 27 pounds, and there was also a clear-cut drop in the serum calcium. On August 16th, 1938, the animal was completely recumbent and unable to feed. In order to prevent it dying in tetany from calcium deficiency (as 110 had done) it was first given a supplement of $CaCO_3$ of 8 grammes per day. This probably prevented death but there was no marked improvement in the general condition. After a further two weeks, as it was still losing weight and the serum calcium had fallen to 4·9, Radiostol was given. The kid then slowly put on weight and the serum calcium rose to normal. Graph I gives the result of the serum analyses of kid 106.

Further consideration of the results of 106 will be given later but it is clear that the basal ration failed to provide sufficient calcium for its requirements.

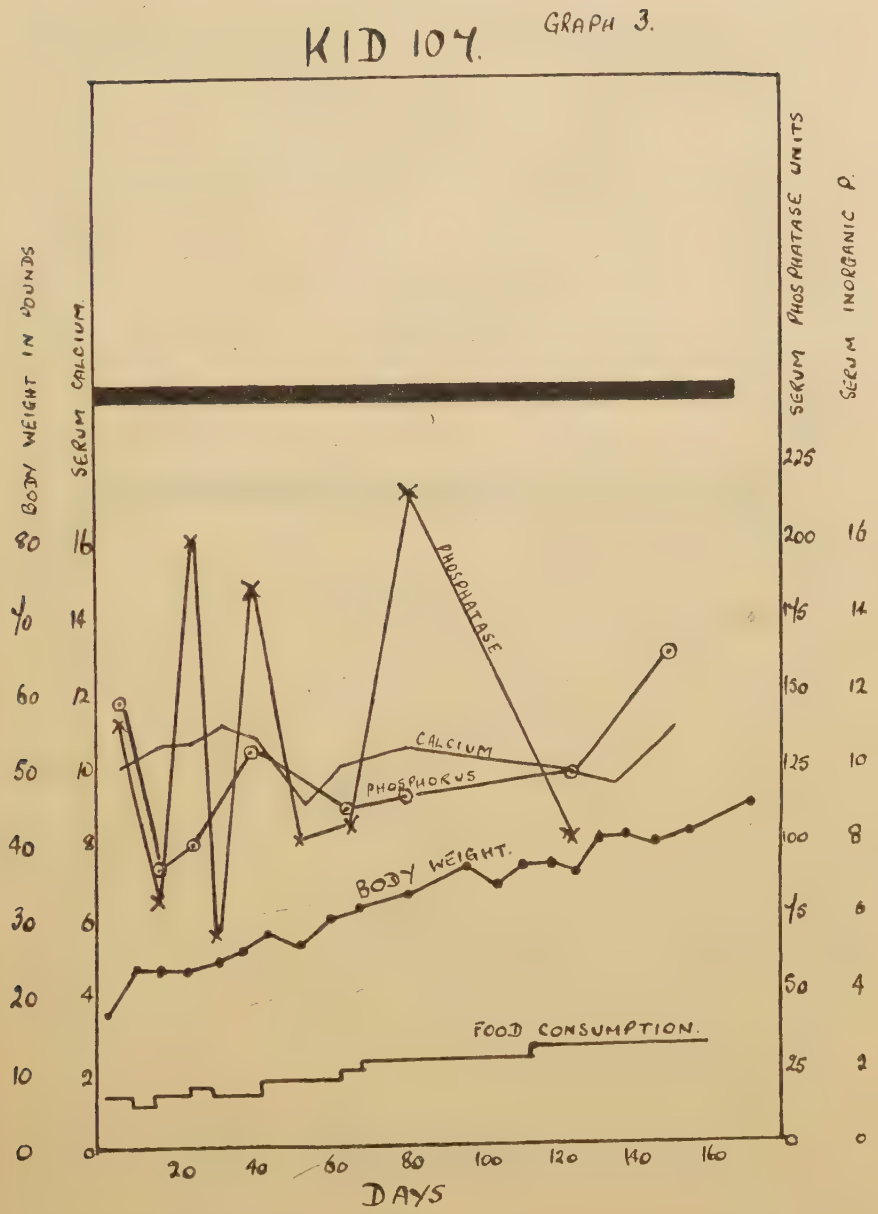
To make certain that the results were repeatable, at the completion of the original experiment another young kid (127) was placed on the basal ration. As was the case with 106 and 110, there was a steady loss in weight, a drop in the serum calcium value to 5·8 milligrammes, and finally marked symptoms of bone derangement. It was killed for histological examination, etc., when its condition was fairly advanced. Beyond changes in the skeletal system, no abnormality was noted in any of the organs.

(*b*) *Basal Ration and Vitamin D Supplement.*—As mentioned above, kid 108 received the deficient basal ration for six weeks and was then given intermittent doses of vitamin D in the form of Radiostol diluted in peanut oil (2,000 I.U. per day) as a preventive. The animal showed a steady increase in weight from 18 to 35 pounds in six months and maintained its condition, while the serum calcium remained relatively steady at a normal level. It did not attain the same final weight as those receiving $CaCO_3$, *i.e.,* 107 and 109, probably because even though the vitamin D had been able to increase the utilisation of the calcium in the ration, the total amount of calcium present was low and probably the limiting factor. The amount of food intake was the same in this animal as in 107 and 109.

It was killed on March 20th, 1939. No changes were detected at the *post-mortem* examination.

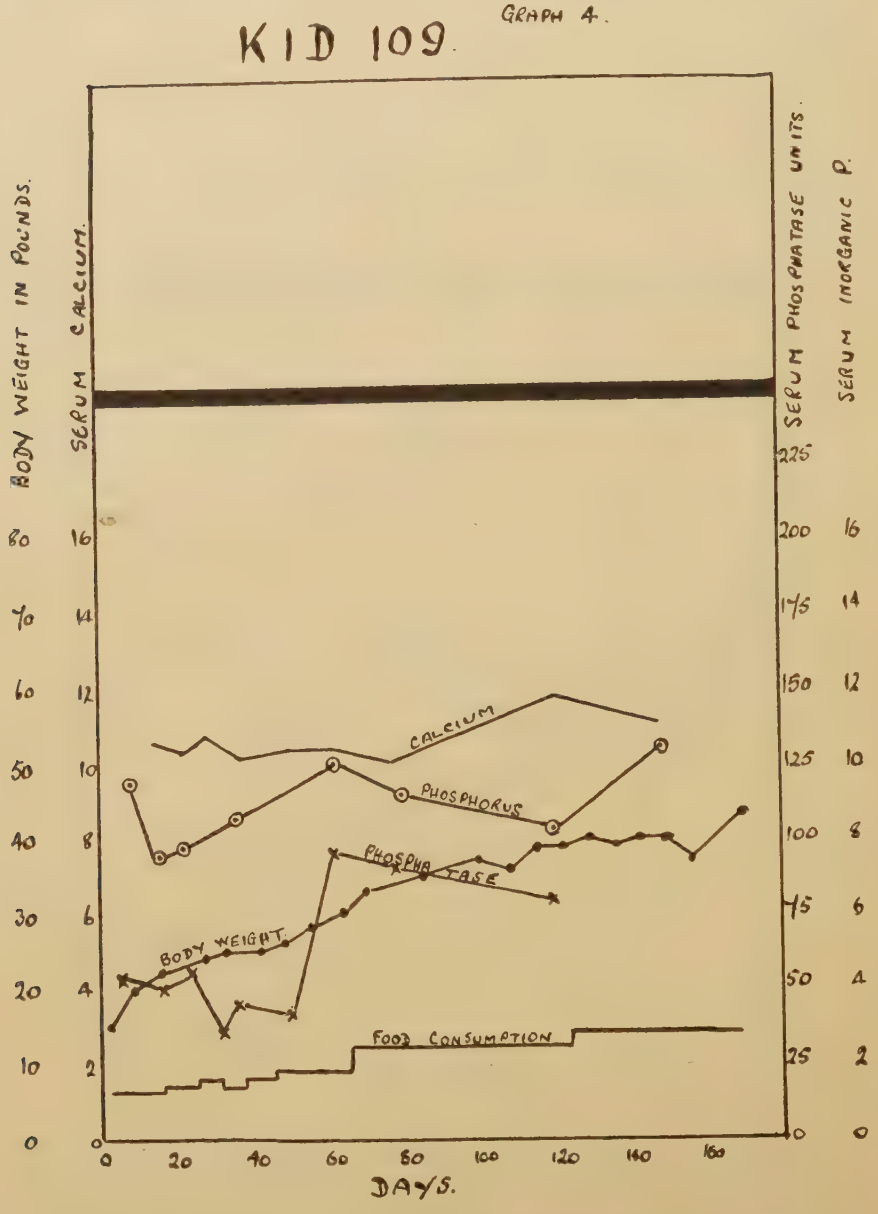

KID 108.
GRAPH. 2
BODY WEIGHT IN POUNDS.
SERUM CALCIUM
SERUM PHOSPHATASE
SERUM INORGANIC P.
PHOSPHATASE.
CALCIUM.
PHOSPHORUS
BODY WEIGHT.
FOOD CONSUMPTION.
DAYS
0 20 40 60 80 100 120 140 160
0 2 4 6 8 10 12 14 16
0 10 20 30 40 50 60 70
0 25 50 75 100 125 150 175 200 225 250 300 350
0 2 4 6 8 10 12 14 16 18 20

(c) *Basal Ration and $CaCO_3$ Supplement.*—Kid 109 received the deficient basal ration and a supplement of $CaCO_3$ of 8 grammes per day. It remained normal and showed a very good increase in growth from 17 to 45 pounds in six months. This result seems to imply that the added calcium carbonate was absorbed and made good the deficiency of calcium in the basal ration. It was killed on March 20th, 1939. No changes were detected at the *post-mortem* examination.



Kid 107 was also given the $CaCO_3$ supplement throughout the experiment, but received in addition intermittent doses of vitamin D. The rate of growth was steady except for a slight decline during a period when the vitamin D was not given. When the vitamin D was again added it continued to grow and attained the greatest weight of any of the goats. The blood serum gave normal calcium

values throughout the experiment. This animal was the only one of the five kids which did not show any abnormal symptoms and is still alive and well.



Effect of Addition of Supplements. Kid 106. The results of the calcium deficient diet on this kid have already been noted. This animal lost weight, became very ill and eventually showed early

signs of tetany. At this stage it was given the $CaCO_3$ supplement alone for 14 days. It continued to lose weight, probably because of a lack of appetite and inability to eat or ruminate, and the serum calcium fell still further. Since there did not appear to be much chance of survival the animal was given the vitamin D supplement. The serum calcium rose sharply to normal and the animal ceased to lose weight; its condition improved slowly but only to a partial extent. Its food intake remained smaller than it had been before it became critically ill. In spite of the added $CaCO_3$, this animal remained unthrifty. On November 26th, 1938, it refused to feed and it was found dead on November 28th, 1938. The *post-mortem* examination showed a heavy infestation with stomach worms of the ostertagia group. There was a well marked, diffuse hæmorrhagic enteritis, which doubtless contributed largely to the death of the animal. It appears that, in spite of the recovery from the tetany, this animal did not make a complete restoration because of the prolonged period of deficiency (see *post-mortem* findings, p. 241).

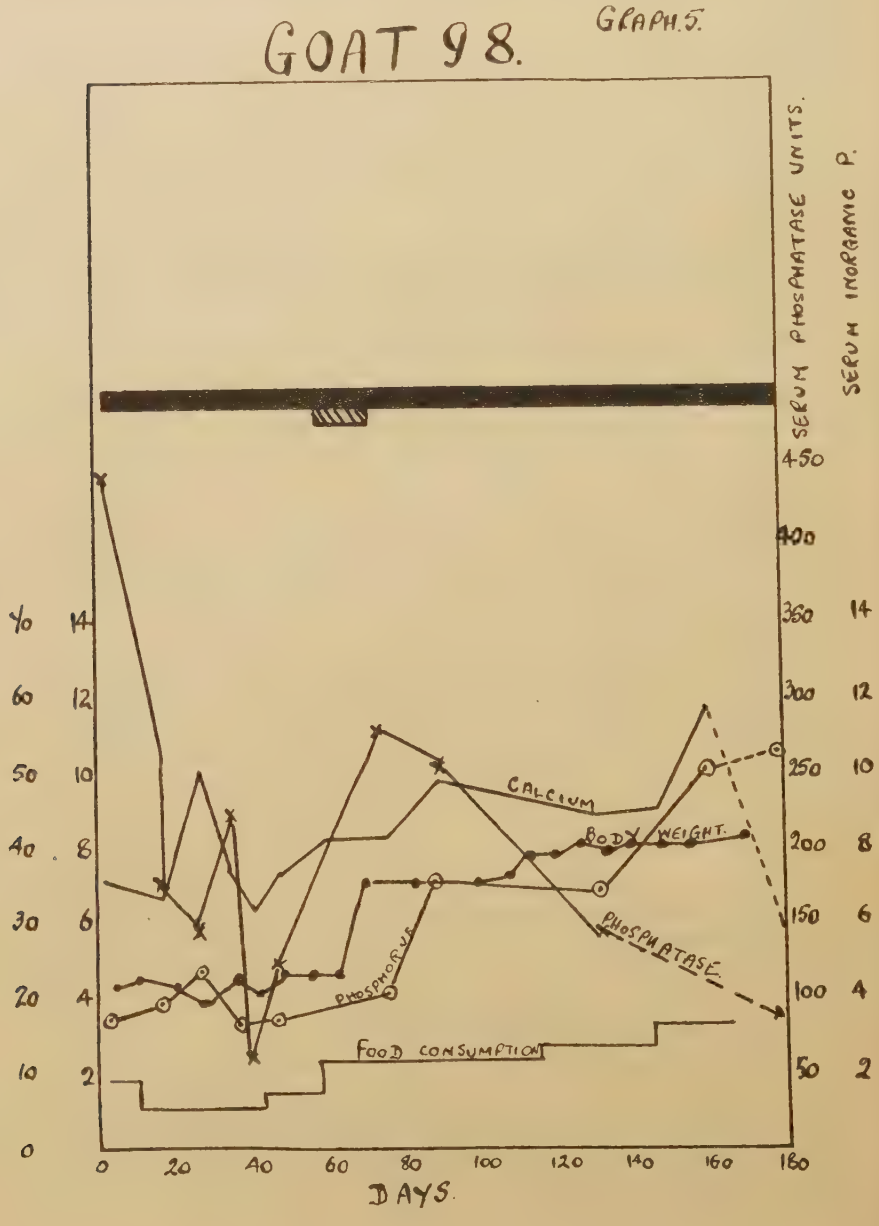
Goat 98, the breeding nanny mentioned earlier, showed a marked loss in weight and deformity of the bones of the head and extremities. Eventually tetany supervened at a stage when the serum calcium was low and serum phosphatase abnormally high. It was then given a supplement of $CaCO_3$ at the rate of 16 grammes per day. Its condition improved slowly and the serum calcium rose but there were intermittent fluctuations. It then suddenly commenced to put on weight, but because the serum calcium was still rather low it was given a vitamin D supplement for 14 days only, receiving 2,000 I.U. per day. Subsequently this animal made a good recovery both from the point of view of its weight, condition and serum calcium.

In the early part of 1939 this animal became pregnant while it was receiving a relatively normal diet of oats, hay, green food and $CaCO_3$ but no vitamin D supplement. At first it maintained its condition but towards the end of term it again showed signs of partial paralysis and eventually slipped a kid. As it seemed unlikely to recover it was killed. Its serum calcium was then 5·8 milligrammes.

At the *post-mortem* examination it was found that the internal organs were apparently normal but there was some distortion of the facial bones and enlargement of the joints which were hæmorrhagic. It may be suggested that two factors were probably responsible for accentuating the hypocalcæmia, *viz.*, the seasonal factor and the condition of pregnancy.

Serum calcium and inorganic phosphorus values in the experimental animals. In animals receiving a diet which contains adequate amounts of calcium, the serum calcium tends to remain at a relatively constant level, provided there is no factor in operation which interferes with its assimilation. This is borne out by the

figures obtained in goats 107 and 109 in which the average value was 10·6 milligrammes, the lowest figure obtained being 9·1 milligrammes and the highest 13·6 milligrammes. For purposes of comparison a determination was made on three normal kids which had been running at grass all the summer and presumably were not suffering from any deficiency. The serum analyses which are given



below agree very closely with those of the experimental kids receiving the calcium supplement.

Kid.	*Ca.*	*I.P.*	*Phosphatase.*
111	9·8	5·6	—
118	10·4	6·5	21
127	9·9	6·6	20
Mean	10·0	6·2	20

On the other hand, wide fluctuations were noted in those animals which were restricted to the low calcium diet. The lowest figures recorded were 4·9 milligrammes (106) and 5 milligrammes (110), while in general the blood calcium was at a lower level than in the controls.

The serum inorganic phosphorus values (I.P.) are never so steady, even in normal animals, as are the calcium values; moreover, the I.P. values are higher in young animals than in adults and may be abnormal in bone diseases. In all the kids there was generally a high I.P. level. In low calcium tetany it is usual to find high I.P., the normal value being 6 mg. P/100 ml. It has been well established in children that uncomplicated rickets, particularly in its early stages, is accompanied by a hypophosphatæmia and a normal Ca value. In experimental animals it has been suggested (Shipley *et al.,* 1922) that two types of rickets occur, the hypophosphatæmia type and the other showing hypocalcæmia with average or raised P. This latter type is associated with tetany. Rominger (1939) gives a consideration of the relation of the low calcium tetany, associated with hypophosphatæmia occurring in children, to the stage of the rachitic condition and the healing process. The fluctuations in value found in these goats may not be clearly related to the clinical condition because it is possible that the blood I.P. in ruminants is normally more variable than in non-ruminants.

Serum Phosphatase.—The serum phosphatase values are difficult to interpret. In ruminants they seem to show greater fluctuations and to be generally higher than in other animals, but since the values of the serum phosphatase in this species are not sufficiently understood this may be a normal factor. In addition, the blood samples were not always taken at the same time of day in relation to food intake, and this may be an important point (Bodansky, 1934). It is not suggested, therefore, that any great stress need be laid on these values or that there is any close correlation between them, the serum calcium and the condition of the animals. It must be pointed out, nevertheless, that the values look so extraordinarily high in some cases as to need a word of explanation. They seem to fall outside the range of those of King and Armstrong (1934) using the same method, but it should be pointed out that in

many cases a dilution of 1 : 10 of the serum was used and in a few cases a 1 : 20 dilution. Some of the phosphatase values were verified by Dr. E. J. King.

Unfortunately, as pointed out, our knowledge of the normal range of serum phosphatase in ruminants is inadequate, so that all that can be said at the present moment is that they seem to indicate gross abnormalities of bone metabolism. In man, the serum phosphatase is high in generalised osteitis fibrosa (von Recklinghausen), osteitis deformans and rickets, but is normal in focal osteitis fibrosa.

Skeletal Changes.—In general, the gross and minute structure of the long bones and jaws of the animals on the basal ration only, resembled the cases of osteitis fibrosa in goats described by other workers.

Two of the goats (106 and 108) had very soft, swollen mandibles which were easily cut with a knife. In addition, 106 showed multiple hæmorrhagic areas. Sections of the mandible of 108 were cut in gum without preliminary decalcification, while a slice of 106 of about ½ cm. thick, was completely decalcified by 1 per cent. nitric acid in one day. In section, the swelling in 108 was seen to be largely composed of very fine fibrous tissue, and the outer layer of compact bone had been entirely replaced by very spongy, cancellous bone. These bone trabeculæ were very small and sparsely distributed. The application of von Kossa's silver nitrate technique to formalin hardened sections indicated that most of these trabeculae were composed entirely of osteoid. The sections of 106 were similar to those of 108, except that there was in addition an enormous amount of brown pigment and extravasated blood accompanied by osteoclasts near both surfaces. The microscopic appearance of the enlarged jaw in 106 was very similar to the appearance of brown tumours described in cases of generalised osteitis fibrosa (von Reckinghausen) in humans. Theiler noticed under the microscope a certain amount of brown pigment in sections from his animals in the tumour-like swellings of the mandibles and maxillæ, but apparently typical brown tumours have not been noted before in animals.

An interesting difference was noted between the jaws of 106 and 108. In 106, although the compact bone had been entirely replaced by very sparse cancellous bone, this was reasonably well calcified and there was not an abnormal amount of osteoid present. This difference can probably be attributed to the fact that, whereas 108 was on the mineral deficient ration the whole time, 106 received $CaCO_3$ for the last two months. This $CaCO_3$ seems to have been sufficient to induce calcification of the osteoid.

As noted by Christeller, the long bones of all our goats, with the exception of 125 (normal control) had a very deficient spongiosa. The diaphyseal spongiosa was very much reduced, particularly in the centre where the intermediate cartilage was in contact with the medullary marrow which thus formed a conelike projection towards

the epiphysis. Another almost constant feature in the long bones was the decrease in width of the corticalis.

The microscopical appearance of the bones from the individual animals may be briefly summarised as follows:—

Goat 97. (Basal ration.) Died in tetany aged one year. The jaws were thin and soft and easily bent. Width of mandible in region of third molar was 15 mm. The long bones were very thin, with a hard corticalis, the width in the case of the femur being 0·4 mm. The spongiosa was deficient but the medullary marrow was apparently normal. The ribs were normal.

Ground sections. A transverse section of the femur shaft. Well calcified, no osteoid, no resorption. Longitudinal sections of the ribs. Junction deep and irregular, poor trabeculæ, osteoid present.

Goat 98. (Basal ration + $CaCO_3$.) Slipped a kid when 22 months old and was killed.

The jaws were slightly swollen but fairly hard, the width of the mandible in the region of the third molar being 17 mm. The long bones were deficient in spongiosa; they were hard with a thin corticalis (width in femur, 1·0 mm.).

Longitudinal sections of the radius and transverse sections of the femur were examined microscopically. The spongiosa was very reduced in amount. The marrow, including that surrounding the trabeculæ, was fibrous and fatty. The intermediate cartilage was very abnormal, broken up into irregular patches and interspersed with bone trabeculæ. Osteoblasts were numerous but there was very little osteoid. The corticalis was thin and abnormally spongy with fibrous tissue in the spaces. Moderate extravasations of blood were noted under the periosteum. Numerous osteoblasts were found near the epiphyseal-diaphyseal junction, but were scanty elsewhere.

Ground sections. Transverse section of femur shaft. No osteoid, very slight resorption at inner border. Longitudinal section of ribs. Junction fairly regular, no osteoid, trabeculæ irregular.

Kid 106. (Basal ration for prolonged period: later $CaCO_3$ and vitamin D). Died of enteritis aged nine months. The jaws, particularly the lower, were very swollen and soft, with dark, hæmorrhagic spots on the surface. The width of the mandible in the region of the third molar was 28 mm.) The mandible cut very easily with a knife. No compact bone was left, being entirely replaced by cancellous tissue. Sections showed the trabeculæ to be thin and sparse and surrounded by a great amount of fibro-cellular marrow. Near the surface there were considerable amounts of dark pigment and a great extravasation of blood. In this area osteoclasts containing pigment were very numerous and trabeculæ very scanty. In most places the osteoblasts were flattened and there was very little osteoid. In general the picture suggested an active process of resorption.

The long bones were hard, with a thin corticalis (width in femur, 1·0 mm.) and a gelatinous, medullary marrow.

Longitudinal sections of the radius and transverse sections of the femur shaft were examined histologically. In the epiphysis the marrow was chiefly fatty, whereas in the diaphysis it was markedly fibrous. The intermediate cartilage had a wavy contour and the cells were arranged in an irregular manner. One very large and several small projections of cartilage had invaded the diaphysis where it joined the fibrous marrow. Many of the newly formed trabeculae adjoining the cartilage at its periphery had streaks staining deeply with hæmatoxylin. The osteoblasts were flattened and there was sclerosis of the periosteum. Definite extravasations of blood were noted in

the periosteum, while abnormal amounts of fibro-cellular tissue were seen between the periosteum and the underlying cortical bone. The corticalis was spongy at the head of the shaft but near the centre it was of the normal compact type with some fibrous marrow. There were relatively few osteoclasts.

Ground sections. Transverse sections of the femur shaft. Haversian canals enlarged in places, no osteoid, no resorption. Longitudinal section of ribs junction irregular, no osteoid, much resorption.

Kid 108. (Basal ration : later given vitamin D.) Killed at one year.

The jaws were considerably swollen and very soft. The width of the mandible in the region of the third molar was 22 mm.) Sections showed that all the compact bone was replaced by cancellous tissue. The trabeculæ were sparse, composed practically entirely of osteoid and surrounded by a large amount of fibro-cellular tissue.

The long bones were hard and slightly bowed. The corticalis was thin (0·7 mm. wide in femur) and the medullary marrow gelatinous.

The parathyroids exhibited a preponderance of ballooned water-clear principal cells, while the thyroids were hyperplastic with small, irregularly shaped alveoli.

Kid 109. (Basal ration + $CaCO_3$.) Killed while pregnant at 12 months.

The jaws and long bones were entirely normal. The width of the mandible was 15 mm. and of the femur corticalis, 2·7 mm. The medullary marrow also presented a normal appearance.

No changes were observed in the parathyroids but the thyroids were hyperplastic and the general appearance resembled that seen in animals on a deficient iodine diet. The cells were of the columnar type and very little colloid was present.

Kid 110. (Basal ration only.) Died in tetany at three months.

The jaws were thin and hard, the width of the mandible being 9 mm.

The long bones were hard. The medullary marrow was very gelatinous and the matrix did not stain well with connective tissue stains. The spongiosa was normal in amount and appearance but the marrow was fibrous. The intermediate cartilage showed a slightly wavy appearance. The corticalis was of normal width (1·1 mm. in femur) but less compact than usual. The spaces were filled with fibrous tissue, which also occurred in parts between the periosteum and the cortical bone. Small amounts of extravasated blood were seen under the periosteum. Osteoclasts were infrequent while osteoblasts were few and flattened.

Kid 125. (Normal.) Killed as control at six months of age.

The jaws were thin and hard (width of mandible, 12·5 mm.). The long bones were apparently normal (width of femur corticalis, 0·9 mm.). Longitudinal sections of the radius were examined histologically. The spongiosa was normal in amount. There was very little fibrous marrow in the epiphysis but considerable areas were noted in the diaphysis. The intermediate cartilage was slightly irregular and some blood extravasation was observed under the periosteum. There was no evidence of osteoporosis. The osteoblasts were normal, and there were relatively few osteoclasts.

Kid 127. (Basal ration only.) Killed at $5\frac{1}{2}$ months.

The jaws were thin and hard (width of mandible, 10 mm.).

The long bones were hard while the medullary marrow was gelatinous.

Longitudinal sections of the radius and tibia and transverse sections of the femur shaft were examined histologically. In the radius, the spongiosa of the epiphysis was abnormally spongy. The trabeculæ were thin with

flattened osteoblasts on the surface and a large amount of fatty marrow. With the exception of a slight irregularity on the epiphyseal side, the intermediate cartilage was practically normal. The diaphyseal spongiosa was also normal in amount but the trabeculæ showed streaks staining deeply with hæmatoxylin. In this region the marrow was chiefly fibrous. Osteoclasts were numerous near the periphery with very little osteoid. The corticalis was normal in width (0·8 mm. in femur). There was a considerable amount of fibro-cellular tissue between the periosteum and underlying the bone, and also in the bone spaces, but a normal cortical structure near the middle of the shaft. Great extravasation of blood had taken place under the periosteum, particularly near the epiphyseal-diaphyseal junction. Many large osteoclasts were seen in this region.

The tibia was similar in appearance to the radius but a gap was noted in the intermediate cartilage in the centre of the bone, and isolated fragments of cartilage from this region were found in the spongiosa of both the epiphysis and the diaphysis. Marked resorption had obviously taken place in the diaphysis. Osteoclasts were numerous and there was also considerable extravasation of blood and some pigment.

Parathyroids and thyroids were normal.

Ground sections. Transverse section of femur shaft. Haversian canals irregular and some enlarged. No osteoid, no resorption. Longitudinal section of ribs. Junction regular but rather wide, no osteoid, trabeculæ small and poorly calcified. Cartilage cells included.

The thyroids and parathyroids of 108, 109, 125 and 127 were examined histologically after fixation in Bouin. The parathyroids examined by us were those which are completely embedded in the thyroid glands. They were of the compact type and appeared to be composed of principal cells only.

In none of our animals was the parathyroid enlarged (*cf.*, von Recklinghausen). The parathyroids of 109, 125 and 127 were normal. Those of 108 were composed practically entirely of the ballooned, water-clear type of principal cell. These cells do not preponderate in normal glands. The fact that the glands of only one of the four goats examined, showed any histological changes, indicates that the parathyroid changes, if any in these goats are secondary to the bone changes and in all probability are relatively unimportant.

Consideration of the Results in Relation to Previous Work.

It is evident from the work which has been done previously on different types of animals that bone metabolism can be grossly affected by the complete or relative deficiency of one of the essential nutritional factors, namely, calcium, phosphorus or vitamin D, and that any deficiency may ultimately limit growth. Animals vary in their response to deficiencies of the different essentials for calcium metabolism. Theiler and his co-workers, who have given a systematic survey of the subject, state that aphosphorosis is the cause of rickets and osteomalacia in cattle and sheep, but that these animals suffer less frequently from calcium deficiency. In pigs, low calcium and low vitamin D are both determining factors in the production

of rickets. In dogs, vitamin D deficiency is the dominant factor, while in equines and goats, calcium deficiency is the usual cause of bone disease.

It follows, therefore, that the form of bone disease which occurs in different animals is related to the animal type as well as to the nature of the limiting causative factor. In humans, dogs, cattle and sheep either rickets or osteomalacia is produced according to age, either of which may or may not be associated with porosis. Similarly, pigs show rickets or osteomalacia on a P deficient diet, but on a Ca deficient diet containing adequate vitamin D are affected with osteodystrophia fibrosa, a disease classed by Theiler with human osteitis fibrosa, but probably different in origin. In horses and goats osteodystrophia fibrosa (Theiler) or osteitis fibrosa is the more common condition. Recently Theiler (1934) has stated that what was hitherto described as rickets in goats is an affection complicated with osteodystrophia fibrosa.

Knaggs (1926) reported osteitis fibrosa in goats and gave a description of the symptoms and condition in his animals, which are similar to those observed in the goats fed on the basal ration in this experiment, particularly with reference to the swollen jaws. It was suggested in this early work that death supervened from starvation owing to the fact that rumination became impossible. Christeller (1924) also noted the occurrence and studied the pathology of cases of this disease in goats, dogs and monkeys. Marek and Wellmann (1931) reported the spontaneous occurrence of osteitis fibrosa in goats receiving diets consisting of hay, oats, bran and sometimes also beet or potatoes. The disease was reported to be associated in one case with rickets, in one with osteoporosis and in two other cases with both rickets and osteoporosis. In two instances the addition of $CaCO_3$ and $CaCl_2$ to the ration, when the animals were severely ill, did not bring about recovery, but this may have been because rumination was difficult. These workers state that it is impossible to cure osteitis fibrosa in goats. Unfortunately they did not inject calcium salts or administer vitamin D in any form, so that it is impossible to say whether the deficiency of calcium in the diet could have been made good in either of these ways. The changes described by them in the jaws and long bones were similar to those seen in our goats, but in contrast we found it possible to overcome the acute symptoms of low blood calcium to a certain extent by the administration of $CaCO_3$ and definitely by vitamin D.

The condition of the animals 97 and 98, namely, swollen and enlarged facial bones, tender hooves, lameness, paresis and anæmia pointed to a condition similar to that already described as occurring in goats and to that reported by Kintner and Holt (1932) in the Philippine Islands as arising in horses fed on a poor (low calcium) native grass and hay. The striking result of the present goat experiments was the reduction of the serum calcium to an extremely low level and the production of tetany, a condition which probably

caused the deaths of 97 and 110. Hitherto, although many cases have been reported and the gross pathology has been defined, we can find no records of systematic blood analyses in this disease. It is known that in horses and cattle, although skeletal disease may be very advanced, it is difficult to reduce the serum calcium level below about 7·5 milligrammes, presumably because of the efficiency of the endocrine mechanism for serum calcium regulation. Groenewald (1938) has shown recently that when horses are fed diets of unsuitable Ca/P rations they readily develop osteitis fibrosa, although there is no change in serum calcium and phosphate. Theiler, Du Toit and Malan (1937) were also unable to produce any marked alteration in the serum calcium level in heifers and steers on calcium or P deficient diets, but this may be attributable to a probable adequacy of vitamin D in the food or environment in South Africa.

In human beings affected with generalised osteitis fibrosa (von Recklinghausen) there is high calcium and low inorganic phosphorus in the serum, while in localised osteitis fibrosa there is no change in serum composition in this respect. Although in von Recklinghausen's disease the parathyroid is hyperactive, it is not definitely known whether this is primary or secondary. In a paper by Hunter and Turnbull (1931), Hunter considers the change in the parathyroid to be primary, whereas Turnbull says it is impossible to tell whether the changes are primary or secondary.

In sheep, on the other hand, Franklin (1934) obtained low serum calcium results accompanied by tetany, when carrying out experiments very similar in type to those on our goats, in which a ration of flaked maize and hay was fed.

It would seem that the sheep and goat are less able than the horse to regulate their serum Ca and inorganic P and this is possibly due to differences in the efficiency of the parathyroid mechanism. We cannot assert, however, that in the goat, because the serum calcium is very low, that the parathyroid is not responding, because it has been shown experimentally that if parathyroid hormone is given in small continued doses to dogs (Morgan *et al.*, 1933; Jaffe and Bodansky, 1930) or to guinea-pigs (Bodansky and Jaffe, 1931) when the calcium content of the diet is low but vitamin D is present, there is no rise in serum calcium in response to parathormone. According to Theiler (1934*b*) parathyroid lesions in domestic animals are rare.

Macroscopic and microscopic examination of the bones and jaws of our experimental goats indicated that we were not dealing with typical rickets. The ribs showed none of the irregularities characteristic of this disease. The long bones were of normal shape, size and hardness, had no thickening of the epiphyses and the intermediate cartilage was of the normal width, although it was frequently very irregular. The Haversian canals were not enlarged: moreover, in none of the long bones was there a preponderance of

osteoid and in many of them very active bone resorption was in progress. This bone resorption seemed to be chiefly vascular in the long bones. Theiler (1934) also found that vascular resorption and not osteoclastic resorption occurred in cases of mineral starvation.

It should be pointed out that the changes occurring in the bones of these goats may have been partly attributable to arrested growth. Du Toit, Malan and Groenewald (1932) state that a deficiency of P affects the growth of sheep very adversely. In the experiments of Bosworth and Stewart (1932), Stewart and Innes (1934) relating to sheep naturally or artificially subjected to diets low in P, there were marked alterations in the bones. It was noted that, at least in naturally produced cases, endochondral ossification was more or less completely suspended. There was some narrowing of the intermediate cartilage, while the trabeculæ of the spongiosa were less numerous and more slender than the normal. Osteoblasts were scanty and small in size, while considerable resorption of the corticalis had taken place, particularly in the deeper layers of the lamellar bone.

In the opinion of these authors, the changes were due to atrophy and osteoporosis of diminished bone activity. It may be suggested, therefore, that both in the sheep and the goat the rarefaction of bone is partly due to inanition and that up to this point the abnormal histological picture is due to the lack of an essential element, *viz.*, either Ca or P. In the case of the goat, the Ca deficiency induces the additional stage of osteofibrosis.

From a consideration of the pathological findings, we must conclude that to give satisfactory bone development in goats as well as to maintain a normal blood composition in respect to Ca and inorganic P, it is necessary to add both $CaCO_3$ and vitamin D to this particular basal ration. While vitamin D addition alone can make the blood picture normal, the basal ration is still inadequate in calcium. We might emphasise the seasonal character of this spontaneous condition of osteofibrosis in goats, a result of calcium deficiency, which has a peak in early spring. The symptoms of this deficiency are accentuated in pregnancy.

It would seem probable, therefore, that the characteristic manifestation of calcium and vitamin D deficiency in goats is osteofibrosis and not rickets. This disease is associated with low calcium, high inorganic phosphorus and abnormally high phosphatase in the serum. The disease when untreated is fatal, the animals succumbing with low calcium tetany (goats 97 and 110) and showing marked pathological changes in the jaws and long bones (osteofibrosis). The parathyroids are normal in size and structure.

Summary.

(1) Goats fed on flaked maize and bran with a minimum amount of hay developed symptoms characterised by loss of weight and appetite, with marked swelling of the jaws, lameness and paresis.

(2) The condition of the jaws and long bones of the animals on this deficient basal ration resembled osteofibrosis.

(3) Blood analyses showed that the serum calcium was very low and the inorganic phosphorus higher than normal, while the serum phosphatase was considerably increased.

(4) Several animals died in low calcium tetany, with serum calcium of approximately 5 mg./100 ml. The state of tetany was relieved by the addition to the basal ration of either $CaCO_3$ or vitamin D concentrates.

(5) The basal ration appeared to be deficient both in calcium and vitamin D, because although the vitamin D addition alone relieved tetany the abnormal bone changes were still evident.

(6) Of all the six kids which were put on to the basal ration with or without supplements of calcium carbonate or vitamin D, only one, which was receiving both $CaCO_3$ and vitamin D, survived for one year and was in good health. All the others either died or had to be killed.

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FIG. 4. Kid 106. (Basal ration. Later $CaCO_3$ and vitamin D). L.S. head of radius $\times 4\frac{1}{2}$. Note irregularity of intermediate cartilage and prolongations into diaphysis where at one point cartilage is in contact with medullary marrow. Also note sparseness of trabeculæ of spongiosa, particularly in epiphysis. Decalcified. Hæmatoxylin and Acid-Eosin.

FIG. 5. Kid 106. Mandible in region of molars $\times 48$. Note the very few trabeculæ, the large amount of fibrocellular marrow, numerous osteoclasts : blood extravasation. Decalcified. Hæmatoxylin and Acid-Eosin.

1. Kid 125 (control animal). L.S. head of radius
. Decalcified section. Hæmatoxylin and Acid-Eosin.

FIG. 2. Goat 98 (basal ration + $CaCO_3$). L.S. head of radius $\times 5\frac{1}{2}$. Note discontinuous and irregular intermediate cartilage, and absence of spongiosa in centre of diaphysis. Decalcified section. Hæmatoxylin and Acid-Eosin.

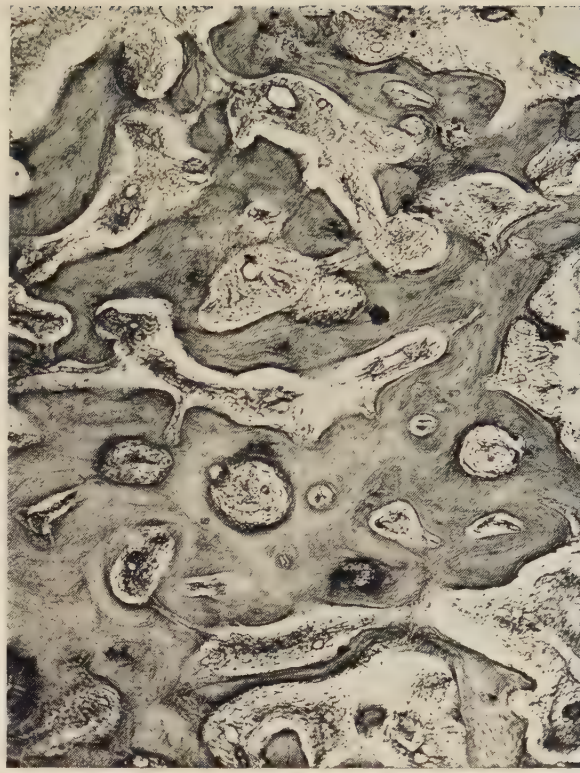

FIG. 3. Goat 98. Same section as Fig. 2. Corticalis of shaft $\times 48$. Note considerable amount of fibrous marrow.

THE INFLUENCE OF VARYING COPPER INTAKE ON NORMAL BLOOD COPPER OF NORTHUMBRIAN SHEEP.

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That copper plays an essential rôle in certain biological processes, notably hæmoglobin formation, is now well established (Elvehjem, 1935). Of recent years, particularly since the Dutch work of Sjollema on " Lecksucht " in 1933, the veterinary significance of copper has been brought into prominence by the further observations of Sjollema (1938), Bennetts and Chapman (1937), and still more recently of Dunlop and Wells (1938). Search of the literature, however, shows that very few studies have been made upon what constitutes a " normal " blood copper level, upon what level can be regarded as indicative of copper deficiency and what response in the blood picture is attendant upon administration of additional copper to the normal daily intake.

Certain investigations into the general physiology of copper from the veterinary aspect have been made in this laboratory over the past few years, and in the course of a collaborative experiment between W. Lyle Stewart and A. P. Ponsford, of Newcastle, and E. L. Taylor and H. H. Green, of Weybridge, on Northumbrian " border pine " of sheep, the results of which will be published later, an opportunity was afforded the present writer of determining the blood copper contents of some 300 ewes whose history in regard to copper intake was known. These data are presented now as providing representative figures for blood copper values of sheep.

Of the few figures in the literature, there may be quoted those of Tompsett (1934), who reported values of 0·163, 0·167, 0·172, 0·180 and 0·156 mg. per 100 ml. for five sheep and those of Bennetts and Chapman (1937) who gave values of 0·087, 0·064 and 0·074 mg. per 100 ml. for three normal Australian sheep and values of 0·004, 0·005, 0·006 and 0·010 mg. per 100 ml. for ewes which had given birth to lambs affected by enzootic ataxia.

Experimental Details.

As full protocols of the main experiment will be published later it is irrelevant for the present purpose to describe in detail the treatment of the various groups. Broadly, the experimental sheep range in age from one to six years and are approximately equally distributed over five groups. The experiment was started in 1936 and additions have been made from time to time, while losses from " pining " and other causes have been made good by replacements from the main flock of the same farm. Lambs of the ewes born since the experiment started have been incorporated into the various groups and these are indicated by the designation of " 1937 " or " 1938 " lambs.

Group A receive ½ lb. of heavily mineralised cake per head per day plus regular anthelmintic treatment (copper sulphate and nicotine) once a month. Group B receive mineralised cake only and Group C are controls, with no additional feeding or anthelmintic treatment. Group D are similarly controls but receive a fortnight's change of pasture three times a year. Group E receive regular anthelmintic treatment. The experimental hillside of about 400 acres is divided into five plots, each group to a plot, and the sheep rely on the grazing as the sole source of their food except for Groups A and B which receive the small supplement of mineralised cake.

The pastures of each plot, sampled in 1936, were analysed for copper and had the following composition, expressed as parts per million on a dry matter basis, Group A, 8 ; B, 7 ; C, 10 ; D, 9 and E, 10. The copper content was thus fairly uniform and in comparison with the data of Sjollema (1938) these figures would not be regarded as abnormal. Assuming a rough figure of 10 p.p.m., sheep eating 3 lb. dry matter per day of the grazing would have a daily copper intake of some 13 or 14 mg. The cake, whose copper content was 52 p.p.m., supplied, at the level of ½ lb. per day, about 12 mg. copper daily so that those sheep receiving cake had almost double the intake of copper of those receiving none. The monthly anthelmintic dose for sheep over a year old was 20 to 30 c.c. of a solution containing 5 g. copper sulphate and 5 c.c. Black Leaf " 40 " per 100 c.c., supplying about 300 mg. copper in soluble form once a month. Lambs received about half to two-thirds of this dosage. This periodic flushing with soluble copper leads to a considerable absorption of copper into the body. Preliminary data on the liver copper contents of sheep from these groups show that much more copper is stored in the liver of those receiving the anthelmintic treatment.

For the present study, copper determinations were made by a photometric modification of the sodium diethyldithiocarbamate method upon whole blood samples, coagulation of the blood being prevented by copper-free potassium oxalate.

The blood copper data are presented in Table I and are arranged according to the ages of the sheep. Of the 306 animals examined all were ewes except the fifteen marked ♂ (wethers) distributed 4, 4, 3, 1 and 3 over Groups A, B, C, D and E, respectively. As mentioned earlier, losses through various causes were replaced by sheep from the main commercial flock, of the same age as those lost. These replacements had been on similar pasture to the experimental sheep but had received periodic changes, so that in effect they correspond to Group D in as far as their " copper history " is concerned. Most of these substituted sheep were put into Group D and in Table I are marked with an asterisk, having received the particular treatment some three or four months preceding the time of taking of the blood samples. Of the extra 1933-34 sheep added to each group, those marked + had been out of experiment for about nine months, being returned on February 14th, 1939. Nine of these fall into the control

group C, and eight into Group E (anthelmintic treatment) and these latter received their anthelmintic treatment a few days previous to the taking of the blood samples. As blood copper, even when artificially raised by oral dosing or by injection, rapidly falls to its normal level (within a few hours) there was no question of an elevated blood copper being found in these sheep within a short time of dosing and, in any case, other experiments from this laboratory show that blood copper of sheep is not raised to any appreciable extent following normal therapeutic dosing with copper sulphate.

The complicating factors of age, sex differences and replacements render difficult an elaborate statistical study of the data obtained and for this it would be necessary to obtain additional data. The purpose of the present paper is to give information on the general distribution of blood copper levels among sheep, and to show the effect of the various treatments the data have been plotted in the form of percentage

TABLE I.

BLOOD COPPER VALUES OF NORTHUMBRIAN SHEEP.
COPPER IN MG. PER 100 ML.

Year of Birth.	*Groups.*									
	C		*D*		*B*		*A*		*E*	
	No.	*Copper*	*No.*	*Copper*	*No.*	*Copper*	*No.*	*Copper*	*No.*	*Copper*
1933	89	·064	121	·064	57	·091	26	·102	153	·066
	90	·085	122	·079	58	·079	27	·082	155	·097
	91	·042	123	·088	59	·097	28	·108	156	·100
	92	·076	124	·064	60	·087	30	·049	157	·117
	93	·040	127	·052	62	·108	31	·088	158	·108
	94	·055	128	·100	63	·059	32	·111	159	·152
	95	·059			64	·120			160	·117
	96	·053								
	Mean =	·059	Mean =	·074	Mean =	·092	Mean =	·090	Mean =	·108
1933-34	531†	·076	546	·076	516	·033	501	·070	561†	·105
	532†	·070	547	·046	517	·049	502	·076	562†	·120
	533†	·035	548	·096	518	·091	504	·082	564†	·082
	534†	·117	550	·073	519	·123	505	·091	565	·088
	535	·035	552	·096	520	·088	506	·087	566†	·114
	536†	·054	553	·075	521	·094	507	·082	567†	·091
	537†	·046	554	·084	522	·036	508	·082	568†	·102
	538†	·082			523	·029	509	·079	569†	·100
	539†	·085			524	·016	510	·061	570†	·082
	540†	·091			525	·111				
	Mean =	·069	Mean =	·078	Mean =	·067	Mean =	·079	Mean =	·098
1934	81	·044	113	·055	49	·114	17	·091	145	·114
	82	·061	114	·099	50	·123	18	·087	146	·105
	83	·105	115	·075	51	·073	19	·093	147	·100
	84	·050	116	·054	52	·041	20	·079	148	·158
	85	·044	117	·072	53	·082	21	·073	149	·146
	86	·064	118	·070	54	·076	22	·087	150	·100
	87	·088	119	·087	55	·117	23	·111	151	·138
			120	·090	56	·034	24	·030	152	·097
	Mean =	·065	Mean =	·075	Mean =	·082	Mean =	·081	Mean =	·120

TABLE I—*continued.*

Year of Birth.	*Groups.* C		D		B		A		E	
	No.	*Copper*	*No.*	*Copper*	*No.*	*Copper*	*No.*	*Copper*	*No.*	*Copper*
1936	65	·073	97	·072	33	·102	1	·097	129	·086
	66	·037	100	·114	34*	·117	2	·096	130	·132
	67	·073	101	·065	35	·108	3	·096	131	·124
	68	·061	102	·086	36	·042	4	·091	132	·138
	69	·029	103	·044	37	·067	5	·117	133	·120
	70	·091	104	·072	38	·126	6	·105	134	·108
	71	·117	105	·076	39	·054	7	·079	135	·102
	72	·064	106	·077	40	·097	8	·085	136	·126
	73	·031	107	·102	41*	·094	9	·096	137	·105
	74	·105	108	·082	42	·067	10	·096	138	·078
	76	·100	109	·069	43	·102	11	·102	139	·117
	77	·049	110	·128	44	·064	12	·079	140	·091
	78	·034	111	·056	45	·100	13	·132	141	·132
	79	·038	112	·039	46	·087	14	·100	142	·102
	80	·036			47	·076	15	·105	143	·102
					48	·140	16	·111	144	·076
	Mean = ·063		Mean = ·077		Mean = ·090		Mean = ·099		Mean = ·109	
1937	223*	·058	241 ♂	·048	208 ♂	·105	200 ♂	·076	232 ♂	·120
	224 ♂	·085	242	·060	209 ♂	·108	201 ♂	·079	234	·111
	226	·043	243	·061	211	·026	202	·085	235*	·083
	227	·105	244	·070	212	·067	203	·073	236*	·152
	228	·064	245	·072	214	·108	205	·079	237	·123
	229	·070	246*	·061	215	·105	206	·079	238	·123
	230	·064	247	·094			207	·064	239	·085
			248*	·036						
	Mean = ·070		Mean = ·063		Mean = ·086		Mean = ·076		Mean = ·114	
1938	328	·032	251*	·083	304	·128	285	·138	265	·132
	329	·117	253*	·097	306	·117	286	·108	266	·105
	330	·085	254*	·073	307	·114	287	·114	267	·105
	331	·091	255*	·110	308	·085	288	·105	269	·095
	333	·079	256*	·128	309	·076	289	·111	270	·152
	334	·070	258	·061	310	·070	290	·111	271	·128
	335	·067	259	·065	311	·100	291	·102	272	·102
	338	·091	260*	·099	312	·088	292	·114	273	·134
	339	·091	262*	·072	313	·061	294	·085		
	340	·082	263*	·082	314	·117	295	·091	275	·123
	341	·043	601*	·084	315	·114	296	·076	276	·077
	343	·043			316	·164	297	·093	277	·094
	344 ♂	·076			317	·091	298	·120	278	·111
	345 ♂	·094			318	·126	300	·096	279	·108
					319	·117	301	·108	281	·114
					323 ♂	·123	302 ♂	·114	282	·164
					324 ♂	·117	303 ♂	·108	283 ♂	·134
									284 ♀	·138
	Mean = ·076		Mean = ·087		Mean = ·106		Mean = ·106		Mean = ·119	
General Mean	·067		·077		·090		·092		·112	
Total No. of Sheep	61		54		64		63		64	
Range of of Values	·029 to ·117		·036 to ·128		·016 to ·164		·030 to ·132		·066 to ·164	

* Substitute put in on 2.11.38. † Returned to group on 14.2.39 after 9 months.

distributions over intervals of 0·002 mg. per 100 ml. The control groups C and D receiving no additional copper have been combined for purposes of comparison and in the charts shaded areas indicate the percentages found with levels below 0·06 and above 0·10 mg. per 100 ml.

Considering Group C first (no treatment), the range of blood copper values is from 0·029 to 0·117 mg. per 100 ml., with a mean of 0·067. Nearly 17 per cent. of the values lie between 0·02 and 0·04, 23 per cent. between 0·04 and 0·06 with nearly 28 per cent. between 0·06 and 0·08 and 23 per cent. between 0·08 and 0·10. Less than 10 per cent. lie above 0·10. Group D, which is similar except for periodic changes of pasture, has a range of 0·036 to 0·128 mg. per 100 ml. with a mean of 0·077, very similar to Group C although a little higher and with the values clustered more closely around the mean. Less than 4 per cent. of the values lie between 0·02 and 0·04, nearly 15 per cent. between 0·04 and 0·06, 43 per cent. between 0·06 and 0·08 and 30 per cent. 0·08 to 0·10. Again less than 10 per cent. lie above 0·10 mg. per 100 ml. For comparative purposes these two groups have been combined as the " controls " of Fig. 1. The mean is 0·071 mg. per 100 ml. with a range of 0·029 to 0·128. Nearly 11 per cent. of the values lie at or below 0·04 and 19 per cent. between 0·04 and 0·06, *i.e.*, about 30 per cent. of the 115 sheep of the two groups lie below 0·06 ; 35 per cent. lie in the range 0·06 to 0·08 and 26 per cent. in the range 0·08 to 0·10. Less than 10 per cent. lie above 0·10 mg. per 100 ml.

Group B which had received the cake allowance in addition to the normal grazing shows a wider distribution, the extreme values being 0·016 and 0·164 mg. per 100 ml. with a mean of 0·090, somewhat higher than the control groups. About 17 per cent. of the values lie below 0·06 (compare 30 per cent. of the controls) but nearly 30 per cent. lie between 0·10 and 0·12, over 10 per cent. between 0·12 and 0·14 and in all nearly 42 per cent. of the values are above 0·10 mg. per 100 ml. Although the range is wider it appears that the effect of the copper feeding generally has been slightly to increase the blood copper values of the sheep by lowering the proportion below 0·06 and raising that above 0·10. The bulk of the values (over 70 per cent.) however lie between 0·06 and 0·12 mg. per 100 ml.

In Group A, which had received the extra mineral feeding in the cake as well as routine anthelmintic treatment, the range of the blood copper values is from 0·030 to 0·132 mg. per 100 ml. with a mean of 0·092 and with most values clustered closely around the mean. About 3 per cent. lie below 0·06 and 35 per cent. above 0·10 mg. per 100 ml. Over 23 per cent. lie between 0·06 and 0·08, 38 per cent. lie between 0·08 and 0·10 and nearly 32 per cent. are from 0·10 to 0·12, that is about 93 per cent. of the values are between 0·06 and 0·12 mg. per 100 ml.

In Group E which received anthelmintic treatment alone the range is from 0·066 to 0·164 mg. per 100 ml. with a mean of 0·112

None of the values lies below 0·06 and 69 per cent. lie above 0·10, with over 38 per cent. between 0·10 and 0·12. In the sheep which had received monthly anthelmintic treatment either alone (Group E) or with the small allowance of mineralised cake in addition (Group A) the blood copper distribution was much more uniform although the mean values were not pronouncedly elevated above that of the control groups. It is interesting to note that the mean value of 0·112 mg. per 100 ml. for anthelmintic treatment alone was actually higher than the 0·092 mg. of Group A receiving supplementary cake as well as

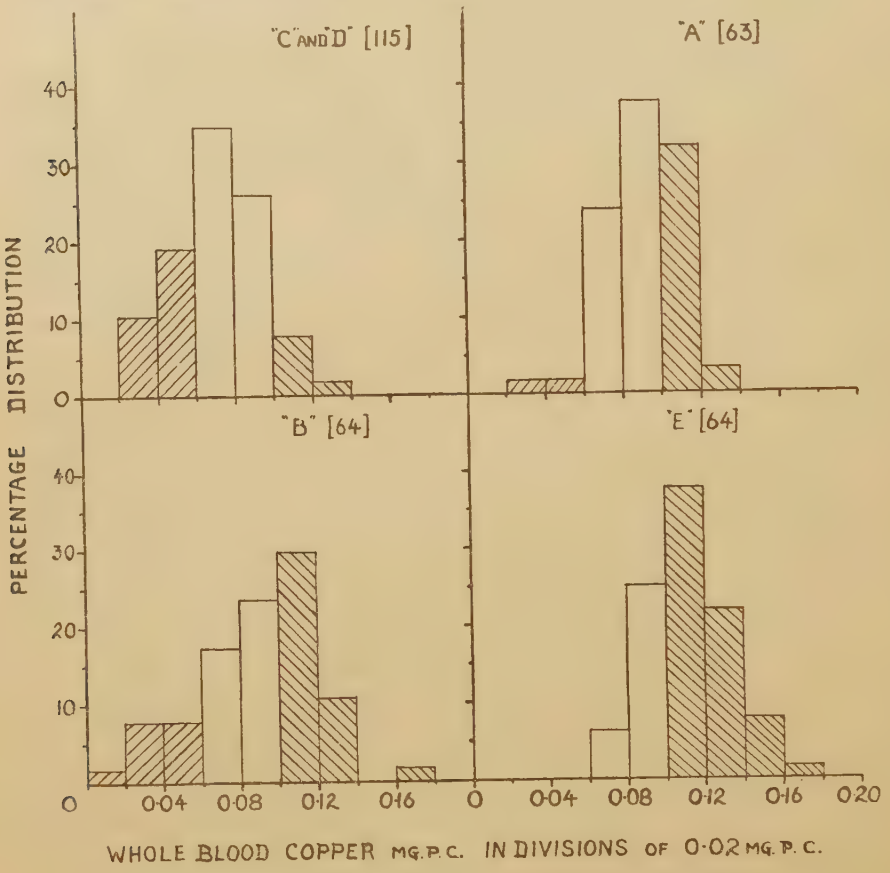

FIG. 1.

Fig. 1. Percentage distribution of blood copper values in sheep.
Treatment differences are C normal grazing; D normal grazing, with periodic changes of pasture; B receiving ½ lb. heavily mineralised cake daily in addition to grazing; A similar to B but with anthelmintic treatment once a month; E monthly anthelmintic treatment only, in addition to grazing.
The figures in brackets indicate the number of sheep in each group.
Groups C and D are combined as controls.
Shaded areas denote percentage values falling below 0·06 or above 0·10 mg./100 ml.

anthelmintic treatment and that this latter figure was about that of the mean of Group B which had received supplementary feeding only. These facts are against any supposition that blood copper levels are materially affected by moderate increases in the intake of copper. The slight general effect of supplementary copper, either by feeding or by routine dosing, upon blood copper level is apparent from Fig. 1.

Considering the 306 values as a whole, the range is from 0·016 to 0·164 mg. per 100 ml. with a mean of 0·088. By far the greater proportion lies between 0·06 and 0·12 mg. per 100 ml. into which range the " normal " figures of Bennetts and Chapman fall. The few figures given by Tompsett are well to the upper end of the range. In this connection Table II shows representative data obtained from animals at this laboratory.

Table II.

Copper Content of Blood of Weybridge Sheep.

Mg. per 100 ml.

1. ·078	7. ·138	13. ·122
2. ·095	8. ·122	14. ·156
3. ·078	9. ·131	15. ·138
4. ·106	10. ·151	16. ·128
5. ·095	11. ·152	17. ·078
6. ·065	12. ·142	18. ·123

Average = ·117

The range for the smaller group of Weybridge sheep is from 0·065 to 0·156 mg. per 100 ml. with an average of 0·117. Most of the figures for the Northumbrian sheep fall into this range, although in the larger series 47 out of 306 or about 15 per cent. are below 0·06 mg. per 100 ml. These are distributed as follows : 2 out of 63 (3 per cent.) in Group A, 11 out of 64 (17 per cent.) in Group B, 24 out of 61 (39 per cent.) in Group C, 10 out of 54 (18 per cent.) in Group D and none in Group E. The high proportion in Group B with blood copper values below 0·06 mg. per 100 ml. (about the same as control Group D) shows that the copper feeding has not had much material effect in raising the blood copper level although the actual figure happens to be less than the 30 per cent. below 0·06 found in the control Groups C and D together.

Preliminary additional data on liver copper distribution throughout the groups show that feeding with mineralised cake has also had relatively little influence in raising the liver copper levels, although the liver figures are higher in those groups which received monthly anthelmintic treatment. There appears to be no connexion between blood copper levels with incidence of " pining " in the respective groups and, although it may be mentioned that the greatest incidence of the disease has occurred in the control groups, the blood copper

levels of those sheep showing clinical symptoms were by no means significantly lower than those of " non-pining " animals within the same group. Many of the sheep with the lowest blood copper levels were as healthy or more healthy than those with higher values and there is no evidence at all that the low values were due to copper deficiency in the diet. It therefore seems that the most logical conclusion is that the tenfold overall variation (from 0·016 to 0·164 mg. per 100 ml.) between individual sheep is a natural phenomenon.

A further point of interest is the variation of blood copper with age. In Table III are summarised from Table I the mean blood copper values in each group, arranged according to age.

Table III.

Mean Blood Copper Values of Sheep Arranged to Age and Treatment. Copper in Mg. per 100 ml.

Age (years).	*Groups.*					*General Mean.*
	A	*B*	*C*	*D*	*E*	
6	·090 (6)	·092 (7)	·059 (8)	·074 (6)	·108 (7)	·084 (34)
5-6	·079 (9)	·067 (10)	·069 (10)	·078 (7)	·098 (9)	·078 (45)
5	·081 (8)	·082 (8)	·065 (7)	·075 (8)	·120 (8)	·085 (39)
3	·099 (16)	·090 (16)	·063 (15)	·077 (14)	·109 (16)	·088 (77)
2	·076 (7)	·086 (6)	·070 (7)	·063 (8)	·114 (7)	·081 (35)
1	·106 (17)	·106 (17)	·076 (14)	·087 (11)	·119 (11)	·100 (76)
General Mean	·092 (63)	·090 (64)	·067 (61)	·077 (54)	·112 (64)	·088 (306)

The numbers in brackets indicate the number of sheep that make up the mean in each group.

It will be noticed from the Table that the general means of all the groups of a particular age are about the same for animals over two years old but that the one year old sheep seem to show a distinctly higher average blood copper level. This is particularly noticeable for those sheep of Groups A and B which had received mineralised cake although the explanation for this is not readily apparent.

Summary.

1. Distribution of blood copper was studied amongst 306 sheep divided into five experimental groups on a " Border Pining " hill-side in Northumberland on which the natural grazing showed a copper content of 7 to 10 p.p.m. on the dry matter.

2. One hundred and fifteen of these animals on grazing only (controls) showed a blood copper range of 0·029 to 0·128 mg. per cent. with a mean of 0·071 and with 61 per cent. of all the values lying between 0·06 and 0·10.

3. A somewhat wider range, 0·016 to 0·164 mg. per cent. but with a slightly higher mean of 0·090 was found in sheep receiving a small supplement of heavily mineralised cake containing sufficient copper to double the daily copper intake. The additional copper,

however, did not bring the lower values up to the mean values of the controls, from which it may be assumed that low values were not due to copper deficiency and that the wide variations shown between individual sheep is a natural phenomenon.

4. Sheep which received monthly anthelmintic treatment with copper sulphate in solution either alone or in conjunction with supplementary mineralised cake showed a more uniform blood copper level but no pronounced elevation of mean value. The fact that the mean value of 0·112 mg. per cent. associated with anthelmintic treatment alone was actually higher than the 0·090 mg. per cent. for the combination of anthelmintic treatment with supplementary cake containing copper is against any supposition that a normal blood level of copper is materially affected by moderate increase of the copper intake.

5. There are preliminary indications that year old sheep have slightly higher blood copper levels than older sheep, the difference being more pronounced in animals receiving copper from birth.

6. Of the 306 Northumbrian animals bled in various age groupings, the overall range of blood copper was from 0·016 to 0·164 mg. per cent., an individual variation of over ten times. Of these 47 showed values below 0·06 mg. per cent. A small group of 18 sheep at the Weybridge laboratory showed a variation of 0·065 to 0·156 mg. per cent., *i.e.*, all within the same range.

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